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PLANT GROWTH-SUBSTANCES



PLANT GROWTH-SUBSTANCES

THEIR CHEMISTRY AND
APPLICATIONS, WITH SPECIAL
REFERENCE TO SYNTHETICS

by

HUGH NICOL

Kéz kezet mos (One hand washes the other)

—Hungarian proverb

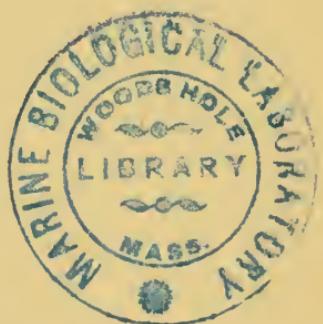
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*Dedicated
in fellowship and with sympathy
to those who in their respective continents
work their way through college
or must carry out their studies while the more
fortunate relax*



PREFACE TO THE SECOND EDITION

This will be found as
Chapter III

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NOTES

This book is based upon a series of articles which appeared in *The Manufacturing Chemist* (Messrs. Leonard Hill Limited, London) in June, July, and August, 1937 (Vol. 8, pp. 175-180, 211-216, 254-257).

References to literature not cited in full in the text or in the list at the end of the Chapter in which they are mentioned, are distinguished by a capital letter indicating the list in which they will be found. This has been done to avoid duplication, while ensuring that each list bears broadly on one subject.

The quotations ascribed to Beaumé are taken from the English translation, by John Aikin, of the second edition of Beaumé's book on Chemistry. (Warrington, 1778).

CHAPTER I

A CHAPTER FOR THE LAYMAN

Vegetables are organised bodies, which extract from the earth the juices proper to their nature. Vegetable substances are much more compound than mineral. Their analysis is consequently more difficult: certain principles, of too great tenuity or volatility, escape us entirely.—BEAUMÉ.

THOUGHTFUL people have always been attracted by the patterned development of a plant. Practitioners of several branches of science allied to agriculture now find considerable interest in the topic of growth substances.

The subject is not really new, as its beginnings can be traced back to some observations of Charles Darwin, but it has recently become popular with remarkable rapidity. This onset of interest is possibly due less to an appreciation of the importance of the subject of growth-regulation than to a widening knowledge of the extraordinary results which can be achieved by the use of some of the growth substances lately prepared artificially. Botanists have long known that, with certain plants at least, it is possible to induce roots to grow from the middle of the stem of a plant, but that it could not be done without some trouble. It is now easy to make roots sprout on stems of almost any soft-wooded plant merely by painting them with something out of a bottle. It is not hard to understand why many laymen, as well as botanists, have had their imagination quickened.

This initiation of rooting on stems and leaves is something more than a laboratory trick, because the same substances that produce roots on leaves will cause roots to appear on cuttings more rapidly than they will appear in the ordinary way after putting into compost. To the horticulturist, therefore, the recent artificial preparations of growth-regulating

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substances represent a real gain of immediate practical importance. Moreover, the possibilities have not been fully explored, and it is confidently expected that the new growth-substances will be useful in grafting and in further types of propagative work in the nursery garden.

The substances are sometimes called growth-promoting substances, but that name is not sound as a general rule. It often happens that a plant that has been treated with one of these substances produces a whiskery out-growth of roots over most of its stem and a great part of its leaves, but becomes dwarfed in comparison with an untreated plant. Dr. H. L. Pearse, writing in the January, 1937, issue of the *Journal of Pomology and Horticulture*, has pointed out that the *amount* of plant-capital is not altered by the application of an artificial growth-substance such as phenyl-acetic acid, but that the *distribution* of the plant's capital is thereby altered. In other words, the more aerial root, the less stem and leaf, the sum of matter belonging to the plant being apparently unchanged. It is, therefore, better to call these substances growth-regulating substances, or, more simply, growth-substances. This book is devoted to an exposition of the chemistry of plant growth-substances; that is, substances, other than fertilizers and nutrients, that can regulate the growth of plants.

The non-chemical reader who glances at the later portions of this book and is affrighted by the array of formulae, and the words, often of more than Johnsonian ponderosity and length, with their uncouth-looking jumble of syllables hyphenated with figures and Greek letters, may think that it is something like English, but what does it mean?

It means, in brief, the story of an attempt to adapt the forces of nature whereby plants grow, not only in size, but in the increase of their several parts—roots and leaves, stems and fruit. It is the summary of an attempt to set our accumulated knowledge to a new field of endeavour: the better control/of plant growth. Firstly, however, it has been necessary to under-

stand how plants grow, and to know what substances affect their different kinds of growth.

The understanding of substances belongs to the science of chemistry, which has built up for itself that queer-looking but exact-meaning terminology which I have already mentioned. The search for answers to the questions that centre around the "how" of plant growth belongs to the science of phytology, or plant physiology, and, in what follows, I have written practically nothing under that head, because I am not a plant physiologist, and don't understand the subject sufficiently to discuss it in detail. However, some hints as to how plants grow can be gained by observing the effect of different substances on plants. The preparation and study of such substances is the job of the chemist.

The substances that can be tested on any one kind of plant fall into two groups: those which the plant can't produce for itself, and those which it can.

Plants cannot produce for themselves the mineral foods they need, and which they normally obtain from the soil; these are such things as phosphates, nitrates, silica, lime, potassium salts, and so on. They also require carbon and water, which they obtain from the air and the soil, and a plant growing from seed has a reserve of food in the seed to start life with.

Given these things, the plant grows. It gets bigger, but it also becomes differentiated, that is, it acquires parts, such as root, stem, and leaf. How does a seedling "know" that it must form a number of parts, all of them necessary, and of several different types, and how does it arrange them into the pattern which is recognizable by us, so that we can say "that is a willow, and that is a tomato plant"?

There is possibly a clue in the activities of some of the substances which the plant produces for itself. A large number of plant-products are structure-materials and reserve foodstuffs, together forming the framework of the plant and its kitchen and pantry. Other substances seem to have a directive or husbanding effect. These latter are the natural growth-regulating substances. They are also called hormones, or "chemical messengers".

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Professor Boysen Jensen, on pages 115-6 of his book on growth-substances, has said: "Numerous experiments have shown that without growth-substances, growth of the shoots of higher plants cannot take place. . . . The increase in cell-wall boundaries is only one manifestation of the fundamental ability of an organism to build itself out of the materials of its environment."

As Boysen Jensen reminds us, there are several modes or kinds of growth. Growth in length or height may be due to the lengthening of a fixed number of cells, without new cells being formed: that is rather like a stretching, only, the force comes from inside, not outside. Growth in length or height may also take place by an increase in number of cells, so that the growing-point is pushed out to make more room for the older tissue. There is also a mode of growth which starts the formation of new sorts of tissue, as when a leaf-bud begins to form. This last kind of growth is due to cell-differentiation.

The effects of growth-substances that have been most intimately studied are those that arise from the first mode, namely, the increase in length of a fixed number of cells. Young seedling oats have become a favourite material for demonstrating the existence of this type of growth. This is partly because it is relatively simple to see what is happening when a certain part (the coleoptile, or first leaf-sheath) of a young seedling oat-plant that has had its tip cut off, has been treated with a trace of the substance that is being investigated. The substance is applied to one side of the cut surface of the stump (the tip, which contains the plant's own shoot-growth hormone, is thrown away). The applied substance causes the cells on the treated side to grow longer (provided, of course, that it is active as a growth-substance). The result is that the leaf-sheath bends to one side in order to relieve the strain caused by the unequal growth. The bending is easily visible.

This is the essence of the oat-test. Oat seedlings are employed because they are cheap, easily grown and handled, are responsive, and otherwise convenient.

The effect of light in causing bending of stems is known to

everybody who has grown plants inside a window-space. For some years, plant physiologists sought to explain the effect of the light, before they suspected the existence of growth-substances. As soon as it became reasonably clear that there was a connexion between light and the elaboration of some growth-material by the plant, efforts were made to isolate in a pure state the substance causing the cell-elongation. Microscopic studies had shown that the observed bending was an expression of greater growth in length of some of the cells on one side of the stem.

The story of the isolation of highly-active growth-substances is an interesting one. The amount of growth-substances in the tips of seedlings was too small to offer any hope of obtaining much of it from that source. After it was noticed that urine had a marked effect in making young plant-cells become longer, efforts were made to obtain the active substance (whatever it was) from urine, which could be obtained easily in quantity. The effort was successful, and enough of a substance, at first called *auxin*, was obtained from urine, to enable its chemical structure to be ascertained. It was found that there were in urine two rather similar growth-substances; the one first discovered is now called auxin-a, the other is known as auxin-b.

In undertaking this chemical investigation of urine, the researchers did not know whether the result of their labours would after all be the finding of a substance identical with or different from the growth-hormone of oat seedlings. Even now it is not known with certainty—only with a very high degree of probability—that an auxin is in fact the natural growth-substance of the seedling oat tip. The presence of auxins in urine propounded the problem as to what they were doing there. It was found that both auxins could be isolated from some vegetable oils, and from malt, so their presence in urine can be accounted for by their being included in our vegetable foods.

Although the formulae of both auxins are known, they have not yet been prepared by purely artificial methods in the

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laboratory. The synthesis of the auxins is a difficult problem in chemistry.

At this point I may make a digression for the benefit of those whose ideas of chemistry are based on the reading of popular novels involving a secret chemical formula. In chemistry, a formula is not a recipe, but is an exposition of the number and kinds of atoms comprising a substance, showing also the way in which the atoms are arranged in relation to one another. Before a natural substance can be imitated in the laboratory, it is necessary to know the exact "configuration" of the molecule of the substance; knowledge of the formula, however, is far from being all that is necessary to enable even an expert chemist to make the substance on the spot. Those who are interested can find the formulae of the auxins on page 113.¹

In urine there is often a third growth-substance, the existence of which was discovered in a curious way by Fritz Kögl and others, of Utrecht. They found that it was not always possible to extract auxin-a from the mixed urines of a hospital. They therefore concentrated their attention on an "especially appreciated source"—the urine of an eighteen-year-old youth, which had been found by the oat test to be four or five times as active as ordinary urines. An isolation procedure applied to this urine led to the production of a very small quantity of crystals of a substance quite different from either of the auxins, though practically as effective in causing cell-elongation in seedling oats. The nature of this substance was a puzzle, but some hints were available, and finally the "new" substance was definitely established to be indole-acetic acid, which was already well known to chemists, but was not then known to be a growth-regulating substance. This shows that if you enter hospital, you never know what purpose you are likely to serve!

Later, indole-acetic acid was found to be present in very

¹The formulae of vitamins B₁ and C can also be found on pages 115 and 116. These substances are now made artificially, and are on sale.

minute amount in yeast, from which it was laboriously isolated after more than a hundred and fifty separate treatments had been made upon three hundredweights of yeast in portions of a kilogram (about two pounds) at a time.

The story of these, and other, discoveries has been riacily told by Dr. Kögl and his collaborators in the "Zeitschrift für physiologische Chemie," vol. 228, of 1934. Regarding the rôle of chance in the sequence of isolation of the auxins and of indole-acetic acid, I cannot do better than give the words (translated, of course) of Dr. Kögl and Dr. Kostermans from page 116:

"It was a lucky accident that from urine, which contained both the growth-substances [*i.e.*, auxin, and indole-acetic acid], we isolated the "real" auxin first. If our attempts to isolate the active substance had first of all led to the finding of indole-acetic acid, probably no one would have taken the trouble to separate the active substance [auxin] existing in the residual liquors. Having acquired the knowledge that yeast and other lowly plants form a substance capable of producing cell-elongation, and identical with indole-acetic acid, one would probably have been content with that, and any variation in behaviour displayed by growth-substances in cereals [oats, especially] would have been put down to the existence of substances only incidentally present. The existence of auxins *a* and *b* would, most likely, have remained unsuspected for a considerable time."

Even now, it is not known why indole-acetic acid and the auxins behave so similarly when applied to the decapitated seedling oat and in some other tests of growth-promotion and regulation. It is usually assumed that substances that display similar physiological behaviour are at least related chemically, but between the auxins and indole-acetic acid there is no obvious similarity whatever. All three substances occur in urine because they are derived from articles of diet, but the origin of urinary indole-acetic acid is quite different from the origins of the auxins.

An important practical consequence arose from the dis-

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covery that indole-acetic acid had growth-regulating properties. Whilst the auxins were new to science, indole-acetic acid, and substances chemically similar to it, have been known for a long time. A number of ways of artificially preparing indole-acetic acid and its relatives were already known before Kögl found the acid to be a growth-substance. It did not take long to test the growth-regulating properties of it and of other substances like it. It was found that a solution of indole-acetic acid, for example, when painted on the stem of a tomato or other soft-stemmed plant caused roots to grow on the stem. From that it was but a step to the attempt to quicken root-formation on cuttings. It was found that indole-acetic acid did considerably hasten the appearance of roots on cuttings put into compost. Hence, it and its relatives are already of practical importance as an aid to horticulturists and gardeners. A number of the artificial growth-substances, including indole-acetic acid itself, are now on sale for horticultural purposes.

If you are a business man or woman, you will perhaps think that here is a field to exploit, by selling manures to which traces of the growth-substances have been added, while you claim remarkable properties of growth-promotion for "Smith's Auxonic Fertilizer". The idea has already occurred to a number of people, but I think that to translate it into practice without more knowledge of the modes of action of growth-substances would be to start at the wrong end. In my view, it is more important to investigate the old, well-tried, organic manures of the farm, and to see whether they contain the growth-substances, and in what proportions. If growth-substances are present in organic manures, a knowledge of their presence and distribution may help us to understand more about soil fertility, and possibly about the healthfulness of foods. Such research work on organic manures has hardly yet begun, but a beginning has been made in America.

Some years ago, the foundation of an understanding of the rotting-down of organic materials was laid at Rothamsted, during an investigation of the decomposition of straw. One practical result was the production of the so-called artificial

farmyard manure—a compost of straw. This artificial farm-yard manure is not artificial in the sense that many purely inorganic fertilizers are. It derives nothing from large live-stock, but is nevertheless a biological product, because many forms of living micro-organisms take part in the rotting. Artificial farmyard manure ("Adco" manure) has been shown to be the equal of farmyard manure as far as crop-producing capacity is concerned. It looks, therefore, as though the biological contribution to fertility is much the same, whether it is supplied by a few large animals or by a multitude of small forms of life.

It is too much to say that the auxins in plants, or growth-substances in general, are the very stuff of life, but the connexion between plant-growth and the natural growth-substances is so intimate that it is tempting to suggest that since the discovery of the modes of action of growth-substances we are a step nearer understanding the mechanism of life itself.

One paragraph more. Once Kögl had found the crystals that turned out to be indole-acetic acid, it was easy, by means of a few comparatively simple chemical tests, to say what the substance was. This was because a great deal was already known about the chemical relationships of indole-acetic acid. The chemistry of indole-acetic acid and of its relatives had been worked out over many years by researchers who were mainly interested in pure, or theoretical—as distinct from applied—chemistry; that is, many of these chemists were interested in chemistry for its own sake. They suspected little of the future importance of their work, but they were broadening knowledge, though, at the time their work was done, a good deal of it must have seemed to have no practical value except, possibly, as an exercise in chemistry. We benefit to-day from the results of the work of these people who laid the foundations of chemistry many years ago. A similar remark would be true of the work of scientists in other fields besides chemistry. That is the reason why I selected as the keynote of this book the proverb that appears on the title-page.

CHAPTER II

ALSO FOR THE LAYMAN

How to use the Commercial Growth-Substances in Quickening the Rooting of Cuttings

Bees burnt to ashes, and a ly made with the ashes, trimly decks a bald head, being washed with it.—[Unconfirmed quotation from N. CULPEPER's *Herbal* (1653); *se non è vero, è molto ben trovato.*]

How to Buy

THE best-known artificial growth-substance is that variously called indole-acetic acid and indolyl-acetic acid, with or without a hyphen and with or without the prefix 3- or β (pronounced *beta*). It is still sometimes called heterauxin. The prefixes serve amongst chemists to distinguish different relatives in a chemical family—all the members of which in the present case consist of the same number of atoms of carbon, hydrogen, oxygen, and nitrogen, but arranged in certain systematically-different ways. Such relatives do not necessarily have a similar action on plants. The prefix 3-means here the same as β . Whenever the prefix is omitted, as it often is in this book, a 3- acid is meant, for little is known about the others, such as the 5- substances. You would be quite safe in asking for simply indole-acetic acid, because only the 3- acid—the one known to be effective in causing rooting on cuttings—is available commercially.

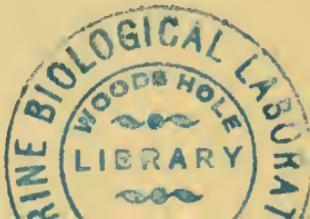
Possibly slightly more powerful than indole-acetic acid as far as root-production on cuttings is concerned, are two other substances. One of them is 3-indole-butyric acid, which is chemically very like indole-acetic acid, being just a little more complex. When speaking of, or placing an order for, this substance, you may again omit any prefix, and ask

simply for indole-butyric acid. The *y* in this word is short, like *i* in *pin*—not like *y* in *why*; and the *o* of *indole* is always short, as in *doll*. The other substance is derived from naphthalene, and is known as α -naphthyl-acetic acid (alpha-naphthyl-acetic acid, the *y* this time being long, as in *why*). There is also a beta-naphthyl-acetic acid on the market. The beta acid is distinctly less useful for most horticultural purposes, so if you decide to obtain a naphthyl-acetic acid for ordinary gardening purposes, you should specify alpha-naphthyl-acetic acid (or, alpha-naphthalene-acetic acid), not, for the present, omitting the alpha part of the name.

All these substances are very expensive, as judged by ordinary standards, and the indole compounds, at least, seem likely to remain expensive for a long time; no cheap method of producing them is in sight. Indole, which may be regarded as the parent of the indole-acetic (indolyl-acetic) group of substances, is used in small quantities by perfumers, and during the Great War it attained the price of £100 (\$500) per pound. This seemed to me to be a record for a commercial chemical. Indole, however, is not a material likely to be bought by the general public even at its present reduced price; yet, until a short while ago, indole-acetic acid was on general sale at a price of about £220 (\$1,100) per pound. It is not very much below that price now, and indole-butyric acid is even dearer.

You may think that at such prices the artificial growth-substances are impossibly beyond our reach, and, indeed, you will see that such prices make chemicals worth literally more than their weight in gold. However, you do not need more than minute quantities, and, since nobody expects to sell indole-acetic acid by the pound, the usual commercial unit of weight for retail sale is the gram: this is a metric unit equal to about a thirtieth of an ounce.

Current prices for some of the better-known pure substances are given on next page from the comprehensive price-list of the British Drug Houses Limited, of Graham Street, City Road, London, N.1:



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3-indole-acetic acid	...	7s. 9d.	per one-gram tube
3-indole-butyric acid	...	10s. od.	" "
α - or β -naphthyl-acetic acid	...	6s. 8d.	" "

Since a usual strength for a solution of these substances is 5 parts, more or less, of the pure substance in 100,000 parts of water, one gram will make 20,000 grams (20 litres) or about sixteen quarts of usable solutions, the cost of a quart thus being pennies only. Small users can buy still smaller quantities: at least some of the substances are sold in tubes containing one-tenth of a gram, and costing about a shilling each.

The Choice of Substance

There is not much difference between the efficiency of indole-acetic, indole-butyric, and α -naphthyl-acetic acids. No one of them is "best" in all circumstances or for all plants, and no definite figures in support of this or that substance can be given. Dr. M. A. H. Tincker in his 1938 paper, of which the reference is given on p. 36, reported that both indole-acetic acid and α -naphthyl-acetic acid brought about the formation of roots on about 55 per cent. of the species he tried, and on about 40 per cent. of those species known to be difficult to root in the ordinary way. These figures will give you an idea of your chance of success in bringing about good rooting of cuttings: with any one species the synthetic growth-substances are about as likely as not to be effective. Even when they bring about rooting of a given species, it may happen that not every cutting of a batch will root. The synthetic growth-substances will not work miracles. What they do is to hasten root formation on some species (after planting out into rooting compost) or to increase the length or number of roots on other species.

Published work with indole-butyric acid has not taken the form that would enable calculations to be made comparable with the above, but it seems to be about as effective as the other two synthetics. There is, however, a growing im-

Rooting of Cuttings

precision that the roots produced on cuttings after a treatment with indole-butyric acid are more desirable in type than those produced by the naphthyl compound at least; in other words, indole-butyric acid scores on quality.

There is one artificial growth-substance which is almost ridiculously cheap, costing only a few pennies an ounce. This is phenyl-acetic acid. It is, however, much less potent than the others that have been mentioned. It often requires to be used at about ten times the strength at which the other substances are used, and even so it produces good rooting with relatively few species. In Dr. Tincker's experiments only about 15 per cent. of species rooted after treatment with phenyl-acetic acid solutions, and as those which rooted were mostly species easy to root, the figure of 15 per cent. compared with about 50 per cent. for the other substances probably flatters phenyl-acetic acid. It has its special advantages, nevertheless, and there is no reason why it should not be tried. It has a delightful rose smell, and if you give it up as a growth-substance you may like to use it to freshen pot-pourri or for other fragrant purposes.

None of these substances, which are called acids for reasons of theoretical chemistry, is corrosive in the ordinary sense. They are all powders, practically bland. They dissolve only to a minute extent in water, though phenyl-acetic acid is soluble enough in water to give a rose odour to a clear (filtered) solution. Compounds of the acids with potash and other bases (*e.g.*, soda, lime) are called salts; they have been used by experimenters, because some of them have the advantage of being appreciably soluble in water. These useful salts are not widely available in the solid form.

How to Prepare

Only very low concentrations of the chosen substance are necessary. As it is difficult to make a very dilute solution straight away, the best way is to make, say, about a one per cent. "stock" solution of the substance in water, by dissolving a known weight of the substance in a hundred times its weight of water. The dearer substances (that is, all of them except

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phenyl-acetic acid) are usually supplied in tubes containing one gram (28·4 grams = one ounce avoirdupois). A measure to contain a hundred grams (millilitres) of water can be bought through a druggist, but three and a half ounces of water will be sufficient approximation to a hundred millilitres.

If the acid, and not a salt, has been obtained, it will be a good plan to dissolve the acid first of all in about five or ten times its weight of alcohol or methylated spirit, which is, however, of no use for the salts. The volume of the solution is then made up with water to the final volume desired for a one per cent. solution.

The stock solution, when not required, should be stored in a cool, dark place in a bottle of dark-coloured glass, because light tends to decompose the substances.

From the stock solution a weaker solution can be made freshly as needed, by adding one part of the stock solution to as many parts of water as are desired. Dilutions of about one part of the original substance in one thousand, ten thousand, forty thousand, a hundred thousand, etc., can be made by adding one part of the stock solution to 9, 100, 400, 1,000 parts of water respectively. These final dilutions will contain respectively 100, 10, 2·5 and 1 part of substance per 100,000 of water. No alcohol or other substance except water is required to dilute the stock solution. Tap water is suitable. Any unused weak solution should not be kept, as it will probably become valueless on standing long.

A smaller measure (to contain 10 millilitres) will be found a convenience both for measuring out the stock solution and for dosing the plants.

To avoid the trouble of making up solutions from the solid, you may also use commercial preparations. These consist of solutions of a carefully-chosen and efficacious synthetic growth-substance already made up into a stock solution. For use, all you have to do is to follow the simple directions. The size of the bottle you buy is not necessarily an indication of what you are getting for your money, since what may seem dear may be merely more concentrated.

To the small gardener an advantage of buying one of the

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reputable commercial preparations is that with each bottle is given a list indicating accurately the special requirements of cuttings of many named plants.

Stock 1 per cent. solutions of named synthetics can also be bought. These are intended for the buyer who for experimental or other reasons wishes to use a particular substance and/or to have it at a known strength.

How to Use

In addition to solutions in water, pastes or salves consisting of the substances mixed with lanolin (wool-fat) can also be obtained or prepared. These are chiefly useful for local applications, such as smearing on definite areas of stems or wounds, and are of little practical value for inducing rooting on cuttings, which is at present the main horticultural utility of commercial growth-substances.

As the whole subject of utilization of growth-substances is new, no definite rules for their use have yet emerged, and to that extent every user must for a time be an experimenter.

The following indicates the general lines of procedure for treatment of cuttings, starting with pure (solid) substances. It may be varied to suit inclinations, and as experience is gained.

For best results, cuttings should bear some leaves.

Divide the batch of cuttings to be treated into three lots. From the stock solution, if that is of indole-acetic acid, prepare three solutions containing, say, about 5, 10 and 15 parts of acid per 100,000 of water. That is, to 10 millilitres of the stock solution add 2,000 millilitres (roughly two quarts) of water, to another 10 millilitres of the stock add 1,000 millilitres (one quart), and to another 10 millilitres add 670 millilitres of water (half a quart, and a bit). (Another way would be to get your pharmacist to make up solutions for you, reminding him that a gallon of water weighs 70,000 grains). Great accuracy is in no case essential.

Plunge the bases of one-third of the cuttings into each of the solutions in ordinary daylight at ordinary temperatures. At the end of 12 or 16 hours withdraw one-third of the cuttings from each vessel, and plant them out in potting

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compost, suitably labelled. At the end of 24 hours withdraw half the remainder and plant them out, and withdraw the last lot at from 36 to 72 hours after the beginning, and plant out. You will have thus nine lots of cuttings, which have been subjected to three concentrations for three periods of time. Concentration of the growth-substance and length of exposure thereto have often to be narrowly judged, but there is a certain latitude—just as in exposing a photographic negative—and at least one lot of your cuttings should have received something like the proper treatment from the suggested ranges of concentrations and times. It would add to the interest if you planted out a few cuttings treated with water only.

If phenyl-acetic acid is used, all the above-mentioned concentrations might be increased about ten times. That is, the amounts of water mentioned should be added to say 100 millilitres ($3\frac{1}{2}$ ounces) of stock solution, instead of to 10 millilitres.

After removal from the solutions the cuttings look just the same as before. All that has happened is that transpiration of water through the leaves has occurred, and some of the solution has been sucked up. Visible roots will not be produced before at least a couple of weeks in compost. The time will depend upon the season of the year, as well as on the kind of plant.

Experience has shown that immersion for 12 or 24 hours in a solution of about 5 to 10 parts of growth-substance per 100,000 of water is about right for several kinds of plants, and this may serve as a rough guide if you are not willing to experiment with several concentrations and times as I have suggested. Immersion in a relatively strong solution for a short time (say 10 parts per 100,000 overnight) seems to be usually better than immersion for a longer time in a weaker solution. On the other hand, an error in judgment of the concentration of a "strong" solution may lead to the preparation of a solution that is poisonous to the cuttings. Be prepared for disappointments; for death of cuttings left too long in the solutions, or in too strong a solution; and for no results, from insufficient

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treatments, and from treatment at an unsuitable time of year, or of species which will not respond anyhow.

Some words by Richard Bradley, a famous eighteenth-century gardener, can fittingly be quoted here:

"And again, the proportions of every Ingredient ought to be as reasonably consider'd, and not to double our Quantities over hastily, because a moderate Proportion has already begun to shew its good Effects; like some unthinking People, who believe, that the more Physick they take, the more healthful they shall be, because they have once been reliev'd from Sickness, by a small dose of it: But a few Observations to an ingenious Man, who loves a *Garden*, will soon bring him to rights in these Matters."

An Excellent Compost

You may like to know of the two standardized composts evolved at the John Innes Horticultural Institution, London, S.W.17. One is for seed sowing and pricking off, the other for potting. These unconventional composts have given superb results, and though the sowing compost (I) does not immediately concern my subject, I give it for the sake of completeness. The proportions are as recorded by W. J. C. Lawrence and J. Newell, of the Institution, in *Scientific Horticulture*, 1936, Vol. 4, 165-177.

	I	II (potting)
	Parts	Parts
Loam	...	5
Moss-peat	...	4
Sand	...	2
Ballast	...	—

It is very important to observe the order of operations exactly, as follows: I. The loam and sand are partially sterilized separately, and the unsterilized moss-peat, super-phosphate and chalk are added afterwards. One and a half ounces of superphosphate (acid phosphate) and 1 ounce of pure chalk are added per bushel of compost.

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II. The loam and ballast are sterilized together, and the sand separately. The unsterilized moss-peat is added afterwards, with the following per bushel of compost: $1\frac{1}{2}$ ounces hoof and horn; $1\frac{1}{2}$ ounces superphosphate; $\frac{2}{3}$ ounce sulphate of potash; 1 ounce pure chalk. The fertilizers must have been finely ground, and all must be mixed thoroughly.

Further information about composts is given in the book by the same authors (Lawrence and Newell): *Seed and Potting Composts, with Special Reference to Soil Sterilization* (London: George Allen & Unwin, 1939. 3s. 6d.).

Note

American readers in particular should note that the treatment of cuttings with indole-acetic acid, indole-butyric acid, indole-propionic acid, and naphthalene-acetic acids (and salts and esters of these) is covered by U.S. Patents respectively numbered 2,129,598; 2,129,599; 2,129,601; and 2,129,600; these were granted in September 1938, and were taken out by A. E. Hitchcock and P. W. Zimmerman on behalf of the Boyce Thompson Research Foundation of Yonkers, N.Y.

CHAPTER III

THE SCOPE OF THIS BOOK

IT is interesting to look back upon some of the comments made by reviewers of the first edition. The book was originally written for chemists, and after the second chapter it assumed and still assumes the reader to have a knowledge of chemistry at least equal to that of pass degree standard. Chapter XIII, which one reviewer justly said could only give the layman a headache, introduces the indole group of compounds, but ignores most of the phenyl compounds, with which the reader of that chapter can be expected to be sufficiently familiar. Few chemists, except specialists in some branches of organic chemistry, know much about the indole (benz-pyrrole) nucleus, though they all know the phenyl ring. Hence the chemical explanations start from the phenyl ring, and no attempt is made to simplify organic chemistry further by introducing explanations couched in terms the non-chemist might possibly understand but of which he would probably lose the thread long before he had an idea of the meaning of the structural formula of benz-pyrrole.

Chemistry, like everything else, has to be learnt; but this book is not the place to teach its elements.

Originally the book contained no plant physiology, and some plant physiologists who reviewed the book apparently concluded that it was therefore a quite unintelligible and peculiarly useless piece of work. Another praised it (on the whole) for what it was, but devoted a good share of his space to regretting that the book did not set forth an explanation of the action of all growth-promoting and growth-regulating substances in every sphere. Such an explanation neither I

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nor anyone else can give, and it is not likely that the ideal will be even approached for a long time.

The book now contains sketchy outlines of some physiological aspects. These have been introduced not as a concession to the over-weaning specialists, but partly because one paradox was not properly explained in the first edition (though no reviewer noticed that), and partly because it is becoming possible to account to some extent for actions which previously were brought about purely empirically.

The difference between just using the synthetic growth-substances to produce a result (such as rooting of cuttings) and understanding how growth occurs is like the difference between pharmacy and physiology. A year or two ago there was practically no knowledge about how the artificial growth-substances produced their results: they were applied like a drug from the outside, produced a certain or uncertain effect, and that was all. There was an increasing understanding of the way normal growth was regulated inside plants by their own natural hormones, but a discussion of these belongs to books on plant physiology.

This book, then, is about drugs that produce, control, or regulate growth in plants. Being foreign to the plant, such applied substances are not hormones, though they are frequently miscalled "plant hormones" or "phytohormones". Both these latter terms connote, and should be restricted to mean, the (natural) hormones of plants, which are the province of physiology and are not discussed in this book, except briefly on pages 53 and 93, and elsewhere incidentally in relation to the synthetic substances.

Since the first edition was written there have been very few changes of significance to the pure chemist, as far as the phenyl- and indolyl-acetic group of substances is concerned. No important new synthetic growth-substance of that type has been prepared; no new method of preparation has been published; and no known substance, belonging to the group, but previously unused, has received any extensive application. The truth of this last can be realized from the fact that the only fresh development worth mentioning from the chemical

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point of view has been the application of phenyl-butyric acid as a reagent in physiological research. This had not been so employed when the first edition was written.

The chief changes since the writing of the first edition have been of horticultural value and interest, with one exception. In 1937 the principal application of synthetic growth substances related to the treatment of cuttings; other developments were foreshadowed, but had not been extensively tested. Since then the treatment of seeds with growth-substances has occupied the attention of a number of workers, the use of the synthetics in grafting has become more than a suggestion, and the questions of bud-inhibition and retardation of flowering have assumed practical importance. These developments are at least sketched in the present edition.

It is hoped, therefore, that the present book may be useful to horticulturists as well as to chemists. The horticulturist will probably get on reasonably well with the book if he skips Chapters X, XIII, and XIV, and some other bits.

Chapter IV on applications of growth-substances to the rooting of cuttings and the production of such freak results as the formation of roots on stems has been maintained in its original form. Some of the work mentioned in this chapter already has not much more than a historical significance, but it has been felt that, historical as they are, such results provide a fairly easy introduction to the more recent practical uses described in later chapters.

The first edition was written in November 1937 (which accounts for some omissions), but could not be published until July 1938. Now (May 1940) the book is being revised and extended with a view of the second edition appearing in the early autumn of 1940. It is not possible to mention every application or to keep strictly up-to-date in a book, but because it is desired to give here as good service as possible, a hint may be offered to those who desire to keep fully informed of developments soon after they are made.

It is possible, though relatively expensive, for an individual to keep up-to-date if he has no access to libraries. Chemists will not need to be reminded of such abstracting journals as

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Chemical Abstracts and *British Chemical Abstracts*. The horticulturist, however, may not know of the monthly *Experiment Station Record* (Washington, D.C., U.S.A.) or of the excellent *Horticultural Abstracts* issued by the Imperial Bureau of Horticulture, East Malling, Kent, England. The latter periodical is a quarterly, and costs 6s. 6d. (about \$1.50) per issue, or £1 5s. od. (\$6.00) per year. To subscribers to certain other publications of the Bureau the price is reduced 20 per cent. The issue for March 1939 contains about 20 abstracts relating to natural and artificial growth-substances, and that number, which may be expected to maintain itself quarterly for a time, gives an idea of the rate at which knowledge of the subject is expanding.

The exceptional development already mentioned as of interest to chemists is the discovery that vitamin B₁ (thiamin, aneurin) is potent as a growth-regulating substance when applied to plants. Vitamin C (ascorbic acid) acts somewhat similarly. Thiamin, at least, may be a serious rival to substances of the indole-acetic acid type at present used in horticulture.

In this book little space has been given to the chemistry of the vitamins. Unlike the indole and naphthyl compounds that act as regulators of plant growth, the vitamins have had considerable publicity given to their chemistry. It has been felt, therefore, that chemists will be satisfied with a few leading references to the synthesis of vitamin B₁, and that it is hardly necessary to say much at this date about the synthesis of ascorbic acid. Probably most chemists will be more interested to learn of the new field for the vitamins—as drugs capable of regulating plant growth when applied to plants from the outside—than they will be to learn much about the artificial syntheses of vitamins, or of their natural production in or by plants and micro-organisms. It does seem, however, that an understanding of the phytological functions of some vitamins, when outside the plant, may have great agricultural as well as horticultural importance.

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FURTHER READING

The history and physiological aspects of hormones particularly, and to a less extent, of synthetic growth-substances, have been well reviewed in the following three books:

Boysen Jensen, P. (1938), *Growth Hormones in Plants*, translated from the German, and revised, by G. S. Avery, Jr. and P. R. Burkholder. McGraw Hill Co., New York and London. 2nd edition.

Schlenker, G. (1937), *Die Wuchsstoffe der Pflanzen. Ein Querschnitt durch die Wuchshormonforschung*. Lehmanns Verlag, Munich.

Went, F. W., and Thimann, K. V. (1937), *Phytohormones*. The Macmillan Co., New York.

A good short introduction for chemists to some physiological matters such as curvature and the oat test (these are mentioned in the next chapter) is the paper by Fritz Kögl, "On Plant Growth Hormones", in *Chemistry and Industry*, Vol. 57, 15th January, 1938, pages 49-54.

A comprehensive review of horticultural aspects of synthetic growth-substances has been provided by H. L. Pearse under the title *Plant Hormones and their Practical Importance in Horticulture*: Imperial Bureau of Horticulture (East Malling, Kent, England) Technical Communication No. 12 (1939); 3s. 6d. post free, or from any bookseller.

CHAPTER IV

MODES OF APPLICATION

Introductory Scientific Work on Effects of the Synthetic Growth-Substances

THE rapidity with which the subject of synthetic growth-regulating substances has developed can be judged from the relative poverty of mentions of these substances in the first edition of Boysen Jensen's book, which was published in 1936. This book is written mainly from the point of view of the academic plant physiologist, rather than from that of the horticulturist. To the chemist it will be of interest mainly on account of the extent of the lists of references to work on the physiological aspects of plant-growth stimulation; most of these, however, are of the "pre-indole-acetic era", having served to convey the ideas of botanists and plant physiologists at a time when the chemistry of plant-growth stimulants was non-existent.

Physiological Methods of Testing

The earliest methods of testing for the presence of growth-promoting substances other than fertilizers were based upon the amount of curvature occurring in a coleoptile (first leaf sheath) of an *Avena* (oat) seedling after the growing tip had been cut off and material containing the substance had been applied to one side of the cut surface. This material was often derived from another growing plant. The test depended upon the stimulation of cell-elongation, and hence of growth on the treated side, so that the cell-tissue became unequally developed, and bending was visible to the naked eye. The method was suited to the detection of very small amounts of active

substance, as a relatively small number of cells were directly affected. In many cases the active substance was that absorbed from a plant-surface by a moist agar surface during a very short time, a small block of the agar being then cut out and applied to the cut surface of another (demonstration) plant, which showed by a bending response that it re-absorbed some of the substance present in the first plant. An *Avena-Einheit* (AE) or *Avena* unit (A.U.) has been devised. Full details of this technique and of its numerous refinements will be found in Boysen Jensen's book.

It was soon realized that urine and the resources of biological and synthetic chemistry laboratories could provide larger amounts of growth-promoting substances than those yielded by a transverse section of a plant. The delicate oat-coleoptile (*Avena*) test has remained in use for purely physiological research, but for more practical purposes it has been succeeded by grosser tests largely based either on root-formation—often in highly unusual positions on the plant—or else on the phenomenon known as *epinasty*.¹ Epinasty is an expression by a mature plant of a set of changes due to unequal development of various parts, so that bending, and sometimes twisting, develops. For example, if one side of a stem or leaf receives more growth-promoting substance than the other, the stem or leaf will bend or curl. The phenomenon of epinasty is thus not different in principle from the simple and measurable bending of a decapitated coleoptile of an oat seedling, but visible epinastic changes in mature plants are complicated by the presence of asymmetric structures of various degrees of rigidity—such as the tapering “veins” in the leaves. For this reason epinastic changes must be regarded as gross qualitative manifestations.

Growth Promotion and Growth Regulation: a Distinction

The production of roots, or, to speak more accurately, the initiation of root-formation, is that property of growth-

¹ Epinasty and its opposite, *hyponasty*, are together known as *nastic* movements or responses. The original papers should be consulted for fuller details, including a discussion of the hyponastic effects, epinasty being taken as the type of both for the purpose of this introductory explanation.

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regulating substances of the most practical importance at the present moment. Before discussing the property of root-initiation the author desires to draw the attention of the reader to the fact that the adjective *growth-promoting* has been twice used in explaining bending tests, but that the term *growth-regulating* has been reverted to in speaking of root-production. This is because at the cut surface of a seedling coleoptile the initiation of cell-elongation is promotion of growth. On the other hand, initiation of root-formation on a mature plant, while leading to the differentiation of new tissue in some places, is often accompanied by diminished growth in other parts, and the plant may even become dwarfed (Hitchcock and Zimmerman, 1935; Pearse, 1936-7). Hence it would be illogical to apply the term "growth-promoting" indiscriminately in alluding to substances other than the nutrients in fertilizers. There seems a need to distinguish, as zoologists have done in their own sphere, between the promotion of cell growth and the initiation of tissue differentiation in plants.

The adjective "growth-promoting" is sometimes applied by physiologists to those amino-acids, without which animal growth (in size) cannot take place. It is doubtful whether the adjective is valid in that connexion, which is mainly foreign to the purpose of this book. Some of the work of R. W. Jackson, and of Berg, and others, had its origin in a study of the necessity of certain amino-acids in animal nutrition.

A method of actually observing the formation of roots was reported by F. W. Went (1934), who found that the number of roots formed on cuttings of sweet pea was approximately proportional to the concentration of rhizocaline ("the root-forming substance").

Hitchcock (1935), of the Boyce Thompson Institute at Yonkers, New York State, caused initiation of roots, epinasty of leaves, and bending and swelling of stems of intact tomato, tobacco, African marigold, and other plants by making local applications of synthetic indole-acetic and -propionic acids. He made the interesting prophecy that "The fact that one

homologue of 'heteroauxin' [indole-acetic acid] was found to be active in causing certain formative responses indicates that possibly other indole derivatives and perhaps other unrelated chemicals might induce one or more of these same responses." It will be seen how abundantly this remark has been justified.

Description of Methods

A description of methods may be quoted from a later paper from the Boyce Thompson Institute (Zimmerman and Wilcoxon, 1935):

"The compounds were used as distilled water solutions or mixed with lanolin (U.S.P.).¹ In a few cases where cuttings were involved dilutions were made with Knop's solution.² The cuttings were placed in vials or flasks so that the basal ends were immersed in the solutions. The water solutions were introduced into the stems and petioles [the stalks of the leaves] by means of glass tubes drawn to a capillary at one end. The capacity of the tubes varied, but held on an average approximately 0·3 c.c. of solution. The capillary end of the tube was inserted into the stem or petiole and left to drain into the plant. There was considerable variation in the length of time required for the tubes to empty. Also the response of the plant varied with the rate at which the substance drained from the tubes.

"Lanolin preparations were applied locally by rubbing the mixture on stems or leaves with a glass rod. . . . The usual concentration range was from 0·01 per cent. to 2 per cent."

Another technique used in later work (Hitchcock and Zimmerman, 1936) instead of injection was to dip a slit-up portion of the stem of an otherwise intact plant into a vial containing solution.

The following list of species of plants successfully used in the experiments reported by Zimmerman and Wilcoxon (1935) showed that the effects of certain growth-substances

¹ The lanolin-smear method was introduced by Laibach (1935).

² Physiologically balanced and isotonic with land-plant cells, Knop's solution is a simple mixture of inorganic reagents in water.

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were not peculiar to a limited range of plants: African marigold, tomato, buckwheat, sweet pea, Windsor (broad) bean, sunflower, Jerusalem artichoke, dahlia, sensitive plant (*Mimosa pudica*), and goosefoot (*Chenopodium album*). A number of other unrelated plants were used in later experiments. Amongst them was the tropical vine *Cissus sicyoides* illustrated in Fig. 1.

Epinasty of leaves was induced by application of the substances in lanolin or by injecting a solution by the capillary-tube method. When the substance was applied in lanolin to a narrow zone around the stem of an active plant, leaves above and below the zone of application showed downward bending. Epinasty of any single leaf was induced by applying the lanolin paste lightly to a small region on the upper side of the petiole. There was considerable similarity in the response of plants to the cyclic compounds and to ethylene, propylene, acetylene, and carbon monoxide.

Tomato plant cuttings 5 ins. long with their basal ends immersed in Knop's solution containing the synthetic substances showed declination of the uppermost leaves within a few hours. The use of Knop's solution does not appear to be essential, as Hitchcock (1935) and others have reported a similar response to aqueous solutions of indole-propionic acid. Zimmerman and Wilcoxon (1935) did not determine the absolute lowest concentrations effective in eliciting this rapid response, but they found that the following low concentrations were effective in that way: Phenyl-acetic acid 125, α -naphthalene-acetic acid 20, and indole-butyric acid 4 parts per million of solution. On the other hand, α -naphthyl-acetonitrile did not cause an evident response in tomato plant cuttings until the second day after its application in solution to the basal ends. Whether applied thus, or injected, or in lanolin, α -naphthyl-acetonitrile evoked final results with tomato similar to those obtained with α -naphthyl-acetic acid.

Growth substances applied to one side of active shoots caused negative or positive bending, according to the concentration of the chemical, and the plant species.

Altogether eight compounds were found by Zimmerman and Wilcoxon (1935) to cause unusual activity such as nastic responses, root-initiation, etc., when applied to sizeable plants; the list has been lengthened since.

Initiation of Roots

All the compounds mentioned in the preceding section, and also anthracene-acetic, fluorene-acetic, and acenaphthene-acetic acids, caused local initiation of roots (adventitious roots) on aerial parts of growing plants of tomato, sunflower, African marigold, Jerusalem artichoke, tobacco, dahlia, and buckwheat. α -naphthyl-acetic and indole-butyric acids were especially effective in initiating roots on *stems and leaves*. The subjoined table, taken from the paper by Zimmerman and Wilcoxon (*Contrib. Boyce Thompson Institute*, 1935, 7, 211), indicates the approximate effective concentration ranges of five of the substances, expressed as percentages in lanolin. Numerical results are, of course, affected by the mode of application, the species of plant, and the type of effect sought.

TABLE—EFFECTIVE CONCENTRATION RANGES OF FIVE SYNTHETIC GROWTH-SUBSTANCES FOR TOMATO, EXPRESSED IN PERCENTAGES IN LANOLIN.

<i>Chemical Substances</i>	<i>Causing Negative Bending in Stem or Epinasty of Leaves.</i>	<i>Inducing Adventitious Roots.</i>
<i>a</i> -naphthalene-acetic acid	0·0100—2·0	0·1—2·0
Indole-butyric acid	0·0100—2·0	0·1—2·0
Indole-acetic acid	0·0003—2·0	0·4—2·0
Indole-propionic acid	0·0250—2·0	1·0—2·0
Phenyl-acetic acid	0·0250—3·0	1·0—3·0
Fluorene-acetic acid	0·0500—3·0	1·0—3·0

This remarkable initiation of roots is well shown by Figs. 2 and 3, taken by permission from the paper by Zim-

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merman, Hitchcock, and Wilcoxon (1936, p. 109). Though this paper was mainly devoted to discussing the effectiveness of esters of the cyclic acids, the mode of root-formation by the acids, esters, and the one nitrile tested was similar. Fig. 2, indeed, presents evidence of the root-initiating capacity of both α -naphthyl-acetic acid (left) and its ethyl ester (right), applied to a leaf and one side of the stem of each plant. Fig. 3 shows an especially beautiful demonstration, the plant on the left being in this case untreated, that on the right having been treated with a low concentration of methyl 3-indole-acetate only ten days before the photograph was taken. The plant shown in Fig. 3 belongs to an uncommon species—*Kalanchoe daigremontiana*—which has the property (useful for teaching purposes) of producing white roots in a dry atmosphere.

The initiation of roots on woody plants such as apple, holly, and *Cupressus* is a problem which has not yet been solved satisfactorily in all respects; there remains considerable scope for experimentation. The lanolin-smear method appears to be less generally effective with the woody plants than with such plants as tomato and *Kalanchoe*. F. W. Went (1934) and Cooper (1935) suggested that the apical ends of cuttings, and not the basal ends, should be treated with preparations of the growth substances, in accordance with the idea that "auxins" can move only downward in stems. Other workers have shown that treatment of the basal ends of cuttings with lanolin, water or dust preparations of synthetic growth substances can induce root formation.

Practical Methods of Using the Substances

With respect to the practical use of solutions of growth substances for inducing root-formation in woody cuttings, Hitchcock and Zimmerman (1936) recommend leaving the cut (basal) ends of cuttings for twenty-four hours or several days in aqueous solutions containing relatively low concentrations in preference to using higher concentrations for periods shorter than twenty-four hours. Desirable concentrations of what they call the three principal substances (indole-acetic, indole-butyric, and α -naphthyl-acetic acids)

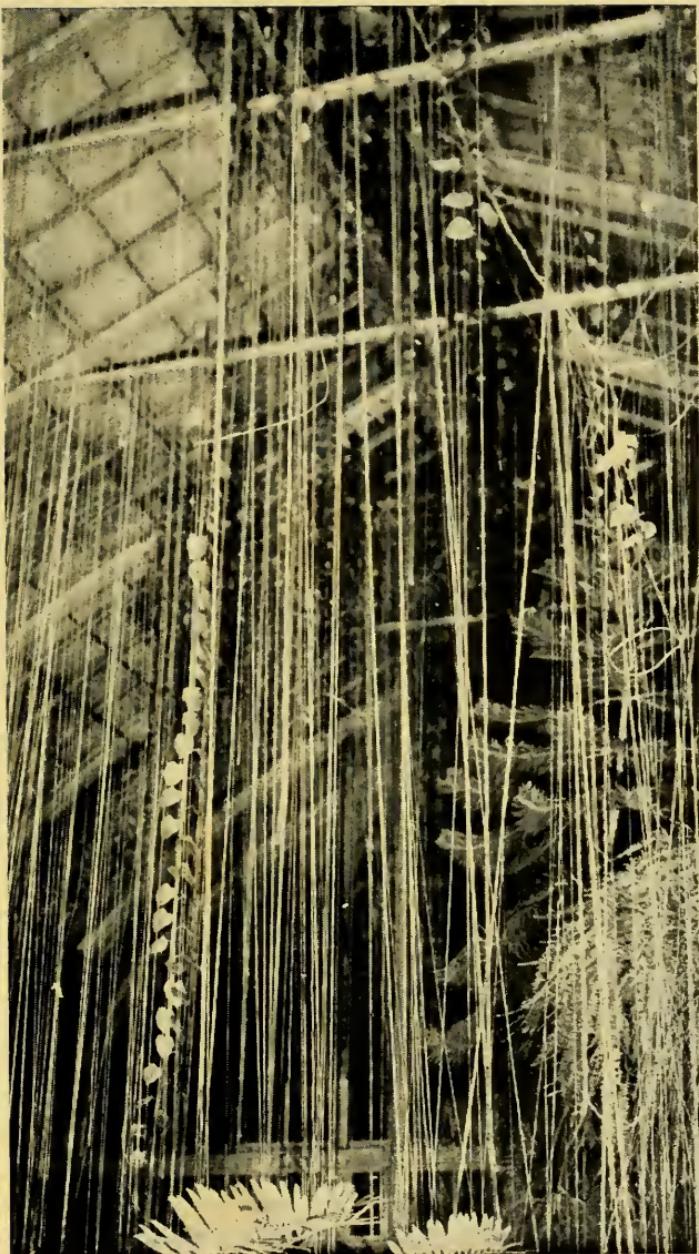


FIG. 1

Root-production extraordinary. This photo shows the natural aerial roots of a tropical vine growing in the Cycad House of the Missouri Botanical Garden, St. Louis, U.S.A. In this greenhouse these roots attain a length of 40 feet, not branching at all unless they strike soil or moist material. The roots here shown owe nothing to artificial growth-substances, and the illustration is given as a matter of general interest.

According to John A. Moore (1933), from whose paper the photograph is reproduced, the vine shown had been in the greenhouse only five years. It was included by Zimmerman and Hitchcock (1935, H) in a series of plants subjected to the action of root-forming substances, towards which it behaved in no unusual way. The botanical name of this vine is *Cissus sicyoides* L. var. *Jacquini*.

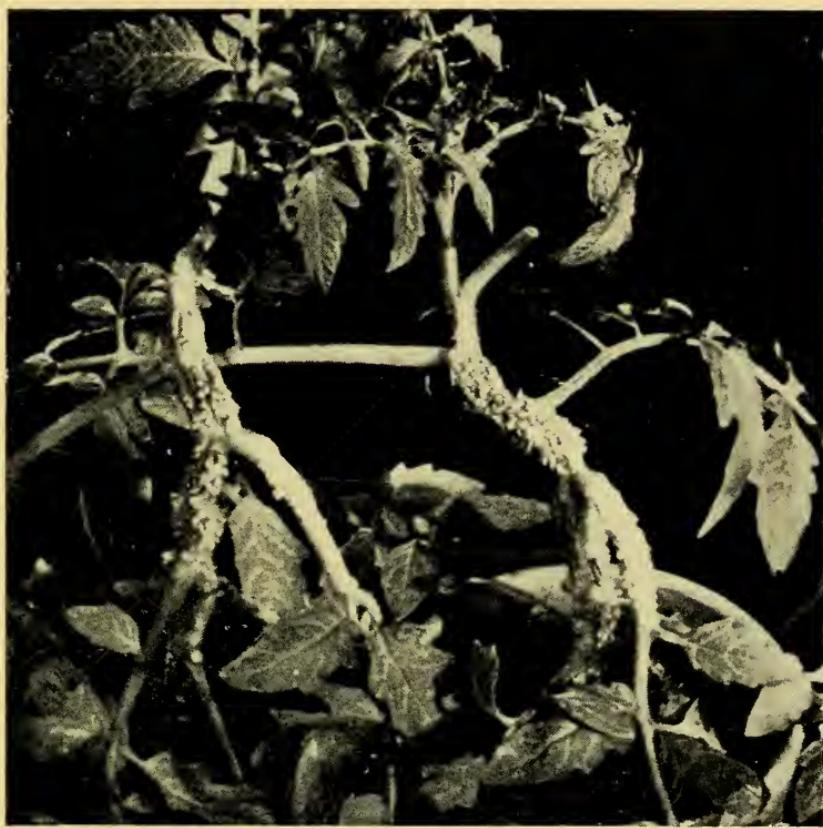


Fig. 2.—Tomato plants, showing coloured roots grown in dry air of the greenhouse, after treatment with lanolin preparations of the growth substances. There are roots on the long stem-like leaf-stalks bending towards the camera, as well as on the true stems. The bending of the stems arose from the treatment on one side. The photographs were taken 18 days after treatment. Left: Leaf and one side of the stem above it, smeared with 0.50 per cent. α -naphthalene-acetic acid.
Right: Similar plant treated with 1.0 per cent. ethyl α -naphthalene-acetate.



Fig. 3. *Kalanchoe daigremontiana*, a tropical "greenhouse" species, here kept under reasonably dry conditions. Left: Untreated. Right: Showing the collar of white roots induced near the top of the stem ten days after local treatment with a lanolin preparation containing 0.05 per cent. methyl β -indole-acetate.



Fig. 4.—Rooting response of cuttings of American holly. The bottom row had had their lower ends dipped for 54 hours in a solution of 10 mg. of indole-acetic acid in 100 ml. of water. The top row was placed in tap water for the same time. All cuttings were then put into a compost of peat-moss and sand for five weeks before the photograph was taken.



Fig. 5.—Two sets of cuttings similar to those of Fig. 4, except that the lower row of cuttings were dipped for four days in a solution of 2 mg. of α -naphthalene-acetic acid per 100 ml. water, the controls being put into tap water for four days.

would be from 1 to 4 parts of the substance in 100,000 parts of water. A small quantity of alcohol may be used for preliminary solution of the substance; the naphthyl-acetic acids are all but insoluble in pure water. After treatment with the water solution for two to four days the cuttings should be placed in a potting compost. The novice should be warned not to expect to see any extensive root-formation at the end of a few days in the solution; the immediate purpose of the treatment is to enable the cuttings to absorb the growth-substance, which will then initiate root-formation while the cutting is in the compost. The formation of roots, however, usually takes place in appreciably less time than when compost alone is used.

Figs. 4 and 5 show respectively the effects of solutions of indole-acetic acid (10 parts per 100,000) and α -naphthyl-acetic acid (2 parts per 100,000) on batches of ten cuttings of American holly. These photographs are reproduced by permission from the paper by Hitchcock and Zimmerman (1936, p. 66). Similar results were obtained with Japanese holly and *Taxus cuspidata* (Chinese yew)—also difficult plants on which to induce rooting with compost alone. It will be noticed that the cuttings that were almost leafless produced few roots, in agreement with the experience of gardeners who have used compost only. Rooting responses of a number of other species of plants are described in the paper.

Japanese maple was one of the few species of woody plants which responded consistently to treatment with lanolin preparations, concentrations of from 25 to 100 mg. of indole-propionic acid per gram of lanolin having to be used. As these concentrations are very much higher than those necessary for the solution method, it appears that the lanolin smear method will be less generally applicable than the solution method. Probably lanolin smears will be restricted to treatment of wounds on the parent plant (as after pruning) to hasten the formation of callus, or else as a technique in suitable cases where it is desired to treat a cutting before removal from its parent. Lanolin smears may

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also be useful in grafting, thus being a refinement on the use of dung. The work of Pearse (1936-7) suggests that spraying may be in some cases at least as effective as lanolin smears.

Cooper (1935), who applied a 1:2000 lanolin paste of indole-acetic acid to a scraped surface at the tops of cuttings, was able to induce formation of roots on leafless lemon cuttings, which do not ordinarily form roots. He described his experience as "very encouraging", and his 1936 paper gives additional results with cuttings difficult to root.

Evenari and Konis (1938, D) whose work is mentioned at length on p. 52, repeat (in agreement with other authors) that there is no general method of applying indole-acetic acid to suit all cases. They point out that a lanolin preparation applied to leafed cuttings may vitiate root-formation, by preventing the intake of water. A related suggestion has been put forward by Jackson (1938), namely, that failures to secure rooting with winter cuttings after treatment with synthetic growth-substances (in solution) may be due to imperfect absorption by the leafless cuttings.

Grace (1937, C) has also obtained excellent root-production on woody cuttings, by dipping the bases of bunches of 50 into a dust bearing 0.1 per cent. of growth substance by weight, shaking off the excess dust, and planting immediately. This technique should be very valuable, and appears to be coming into general use.

The substances tried by Grace in his very promising study included indolyl-acetic, -propionic, -butyric, and α -naphthyl-acetic acids, and some salts and mixtures of these. A few additional details of this procedure are given in the next two chapters (pp. 37, 42, 46).

Pearse (1936-7) has examined the effects of occasional and repeated spraying with solutions of phenyl-acetic and indole-butyric acids, having investigated the action of phenyl-acetic acid at greater length than any other worker. Epinasty of leaves of young tomato plants was clearly visible within thirty minutes of spraying with a 0.1 per cent. solution of phenyl-acetic acid, which could also cause the production of roots from the stem.

He wrote:

"By the spray method of applying the growth-substances to the aerial parts of plants, uniform entry of the substance is secured so that no bending of the stem occurs. . . . The experiments described have shown that with the treatment given the growth-substances cause no increased growth of the plant as a whole, but exert, on the other hand, a reducing effect. With the appropriate concentration, this reduction is minimal.

". . . The treatments seem to have a very large effect on the way in which plant capital is distributed. The substances do in fact appear to control the amount of plant capital that shall go to the making up of each particular organ, without altering the total amount of plant material."

Templeman (1938) obtained decreases, but no increases, in dry-matter production of plants grown in pots of sand, by spraying the foliage or watering the sand with indole-acetic or α -naphthyl-acetic acids, ascorbic acid (vitamin C), or skatole.

Uptake from Soil

Hitchcock and Zimmerman (1935) reported that the addition of synthetic growth substances to soil induced all the responses previously observed by them after applying the materials to the aerial parts of plants. There were differences in the extent and rapidity of response to substances applied to soil, these differences depending not only on the species of plants, but also upon the type of growth, magnitude, and environment of the plant. As before, only a few results can be quoted here. The plants were grown in 4-inch pots holding the equivalent of about 450 gm. of air-dry soil. The solutions were made by extensive dilution with water of an alcohol solution of each compound. Phenyl-acetic, phenyl-propionic, indole-acetic, -butyric, and -propionic, and naphthalene-acetic [? α] acids were tried.

Epinasty (downward bending) of some or all leaves of

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tomato plants from 4 to 10 or more inches high occurred after treatment of the undisturbed soil with any of the six acids except phenyl-propionic. Pronounced responses were often displayed within two hours of the application of the solution: plants about 5 inches high required only about 3 mg. of the substance in order to show the effect, but larger and older plants required 10 to 20 mg. On the whole, the indole compounds were more effective than the phenyl; and toxic effects were observed after application of more than 20 mg. of the indole compounds [to the youngest plants?] or about 50 mg. of the phenyl compounds.

The responses of tobacco plants were generally similar to those exhibited by tomatoes, except that no noticeable response was evoked in tobacco in less than three to six hours.

Root growth from the stems of tomatoes was essentially the same after application of the active substances to the soil as has already been described for local applications. However, "Roots induced by phenyl-propionic acid (applied to soil) appeared sooner and in larger numbers during the first eight days than did roots induced by other compounds. Phenyl-propionic acid was the only compound to induce many roots to appear from parts of the stem that did not exhibit noticeable swelling or other type of proliferation. Naphthalene-acetic acid was the most toxic to root systems in soil. . . . In addition to root growth from the stem of the tomato, roots also appeared from the petiole [leaf-stalk] and along the entire midrib of the middle leaves. This type of response was induced by indole-propionic acid (27 mg. per pot). . . . Indole-butyric acid was especially effective in causing roots to be initiated in stems of tobacco plants 10 to 12 inches in height." Optimum initiation of aerial roots in tobacco resulted from the addition of 27 mg. of indole-butyric acid to the soil.

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The actual or possible effect of a number of factors that may affect the production of roots on cuttings treated with synthetic growth-substances has been well reviewed by B. Hubert, J. Rappaport, and A. Beke (*Meded. Landbouwhoogesch. OpzoekSta. Gent*, 1939, **7**, 1-103), who have compared most of the methods of application. They appear to prefer the dusting technique, with charcoal as the dust.

CHAPTER V

RECENT WORK WITH CUTTINGS

(References *B* on pp. 35-36)

APPLICATIONS

Ordinary woody cuttings

EFFECTS of natural and synthetic growth-substances applied to cuttings of a large number of species of shrubs and plants of horticultural importance have been described by Tincker (1936, 1938). Amongst the illustrations given in Tincker's second paper is one showing roots induced on cuttings of *Viburnum carlesii*, perhaps the most deliciously fragrant-flowered of all shrubs. It is hoped that use of the new synthetic growth-substances, by cheapening the cost of plants, will enable this species to be more widely grown.

Tincker's first paper (1936 *a*) is a review which may be commended to those interested in the history of the use of growth-substances on plants. His second paper (1936 *b*) describes his own experiments; he used synthetic phenyl-acetic, indole-acetic, and α -naphthalene-acetic acids, and an extract of urine (the active substance in which was mainly "heteroauxin"—indole-acetic acid), in the form of lanolin pastes of various strengths. He found tomato and *Pelargonium zonale* useful as test plants for the smear preparations. With some woody and "recalcitrant" species the lanolin-paste method of application was ineffective.

However, aqueous solutions of indole- and α -naphthalene-acetic acids were strikingly effective in inducing rooting to occur in cuttings of woody plants such as holly. Tincker's control cuttings, and those that had been treated with the active solutions, were planted in sand, not compost; other-

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wise his technique was similar to that used by the Boyce Thompson Institute workers.

As has already been suggested on pages 12-17, the artificial growth-substances should be regarded only as an *aid* to propagation, for they will not infallibly compel cuttings to form roots. Doak (1939) has recommended that cuttings intended to be treated with synthetic growth-substances should be taken at the time of year and condition of wood most likely to lead to success without any treatment. Two commercial preparations were found by Johnston (1939) to be of little value in the rooting of hardwood blueberry, which is known to be difficult to propagate in the ordinary way.

Pearse and Garner (1937) have applied the solution-technique to accelerate root-production of soft-wood cuttings of plum and pear rootstocks; immersion of the bases for twelve hours in a solution containing 30 to 40 parts of α -naphthyl-acetic acid per million of water markedly improved rooting. With fig and blackcurrant cuttings, accelerated rooting was also obtained.

Van der Lek and Krijthe (1937) studied the effects of indole-acetic acid solutions on the rooting of plum, quince, and other cuttings. They used the East Malling types A and B (plum) and A, B, and C (quince). The various types of one species responded differently to a given concentration of substance or time of treatment. These workers are the only ones who have measured the actual uptake of solution (and, consequently, of growth-substance) by cuttings, and it appears from their account that the same dose can produce different results, depending upon the species and type of plant, and upon concentration of solution and time of administration. It was confirmed that, in general, a relatively high concentration over a short period of immersion is best. This paper offers several points of novelty, and is of considerable practical interest.

As an example of the variability encountered in treatment, the following fairly full abstract may serve (Komisarov (1938): "Application of growth-substances to increase the rooting capacity of woody species and shrubs"):

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Results with indole-acetic acid (about 0.005 per cent. solution for about 36 hours): 50 to 100 per cent. rooting with 16 out of 24 trials on 18 species. No root-formation with *Pinus sylvestris* and *Thuja occidentalis* 6-7 years old, but 92 per cent. of *Thuja* cuttings rooted if taken from trees 20 years old. In general, the treatment gave (1) greater percentage of rooting; (2) greater root system, especially pronounced with *Acer dasycarpum* and *Ribes rubrum*—most cuttings apparently leafless at the start; (3) earlier rooting—mostly 2-6 days' gain, but up to 16 days with *Thuja occidentalis* of age not stated. Most cuttings were made in summer. *Populus alba* showed no significant difference in the behaviour of winter and summer cuttings, but otherwise the data were insufficient for forming an opinion as to the advantage of taking winter or summer cuttings. Treatment was 12-36 hours for summer cuttings; 20-48 hours for winter cuttings.

Phenyl-acetic and phenyl-propionic acids behaved similarly to indole-acetic acid, but had a less pronounced effect. Summer and winter cuttings of *Populus alba* and *Ribes rubrum* responded readily to the phenyl compounds, but other species did not. (This author is almost the only one who has tested the rooting effect of phenyl-propionic acid.)

α -naphthyl-acetic acid acted similarly to indole-acetic acid on *Populus alba*, *Quercus pedunculatus*, and *Ribes aurea* (the last, however, not having been treated with indole-acetic acid), but had no effect on *Larix sibirica*: on which indole-acetic acid caused roots to form on 26 per cent. of not-woody cuttings and on 6 per cent. of woody.

No satisfactory theory was advanced to account for the difference in responses. Komisarov refers to his 1935 work with *Salix caprea* and *S. cinerea*, wherein he obtained 40-70 per cent. better rooting after treatment with urine, and maize flour "auxins".

A modified treatment of cuttings has been announced by Amlong (1938). He placed vine cuttings (*Vitis vinifera*) in a warm bath for 24 hours, before transferring them to aerated tap water, in which they were kept five weeks. The maximum number of roots thus produced without any artificial growth-

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substance was obtained when the first bath was at 25° C. Addition to the bath of small amounts of one-thousandth or one-hundredth normal¹ solutions of indole-acetic acid markedly increased the number of roots formed under the same conditions.

Forest Trees

Some work with woody cuttings has already been cited.

Only two other papers will be mentioned here. They are given as illustrations of the type of work that has been done, of the variability of the results obtained, and of the fact that generalization is not possible. See also p. 39.

Snow (1938) reported that (1) dormant cuttings of trembling and large-toothed aspen (*Populus tremuloides* Michaux and *P. grandidentata* Michaux) can be rooted to the extent of at least 65 per cent. (of cuttings treated) by dipping them into a solution of 10 milligrams of indole-butyric acid per litre (one part in 100,000) for about 27 hours; (2) maximum rooting is obtained when dormant cuttings of these species are taken just before the buds are beginning to burst in the spring; (3) abundant callus is not indicative of potential root-formation; (4) new hybrids of white poplar treated similarly behaved variously, some rooting to the extent of 70 per cent., others not at all.

Afanasiev (1939) tried the effect of indole-butyric acid on the rooting of greenwood cuttings of deciduous trees, using two species each of *Betula*, *Acer*, and *Populus*. No rooting was obtained with hard maple and aspen, but in four other species 30-60 per cent. of the plants rooted in about 7-11 weeks after treatment with a 2 in 100,000 or weaker solution for 6 to 24 hours.

Sub-tropical Species

Since most of the work on the applications of synthetic growth-substances has been done in temperate regions, many workers in warmer regions will be interested in this section.

¹For explanation of the term *normal*, see p. 55.

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Traub (1938) (of Orlando, Florida) has reported tests of over a hundred chemicals on the rooting of sub-tropical plants. Most of the reagents he tried are not usually regarded as growth-substances, and many of those he used have not been tested by other workers. A number of substances that gave some improvement of rooting (over controls) of *Passiflora quadrangularis* or *Bignonia venusta* are listed in his paper; most of them were inferior to indole-butyric acid in 0·02 per cent. aqueous solution, even in qualitative tests. Salicylidene acetamide (quarter-saturated solution), and tetrafurfuryl alcohol and sodium naphthyl-4-sulphonate (both in 0·01 per cent. solution) were found to have an approximately equal efficacy with indole-butyric acid within the scope of these rough tests. Amongst substances of the more customary class, phenyl-acetic acid, diphenyl-acetic acid, indole-3-n-propionic acid, and ascorbic acid, were tried; they were less useful than the indole-acetic, indole-butyric, and α -naphthyl-acetic acids, but it is not clear whether they were found by Traub to have any value at all.

Many plants (listed in the paper) were considered from the standpoint of their suitability as test objects, and the outstanding ones for sub-tropical use were found to be *Passiflora* species, especially *P. quadrangularis* and *P. ligularis*. Cuttings of the former can be used in the upright or inverted positions. Inversion has the advantage that the controls root late and sparingly. Cuttings were made with the major portion of one internode and one node with leaf attached.

Though reported in 1938, this work was begun in 1933, that is, prior to the identification of heteroauxin as indole-acetic acid. In another paper Traub and Marshall (1937) briefly reported on the rooting of papaya cuttings (*Carica papaya*); they have worked with three *Carica* species.

Gillett and Jackson (1937) and other workers have obtained encouraging results with rooting of cuttings of *Coffea arabica* treated with 10 parts of indole-acetic acid per 100,000 of water, and with appropriate dilutions of a commercial preparation.

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Non-Woody Plants

Demonstration experiments with some non-woody plants have already been mentioned; these were performed to test the efficacy of the then new synthetic growth-substances rather than for purposes of propagation. Recently Nowosad (1939) has described some preliminary tests with indole- and α -naphthyl-acetic acids in the rooting of cuttings of some forage plants, and has concluded that the synthetics may be of value to the plant breeder. It may be recalled that the planting of alfalfa (lucerne) on the field scale from cuttings was practised experimentally in England in the eighteenth century.

The optimal treatments for alfalfa and clover were: (1) 50 parts per million in talc, applied to the fresh scar of cuttings; (2) dipping basal ends 12 hours in 5-50 p.p.m. solution; (3) feeding 10 p.p.m. dissolved in nutrient culture solution.

No satisfactory rooting of timothy (*Phleum pratense*) was obtained. It is pointed out that the degree of success with plants reacting positively is influenced by such factors as temperature, humidity, and light, in the greenhouse; degree of maturity of cuttings; pH and chemical composition of the medium in which the cuttings are planted—as well as by the kind of growth-substance chosen.

See also the work with kudzu (*Pueraria hirsuta*) mentioned on p. 45.

SUBSTANCES

Solvents

As already mentioned, the potassium and sodium salts of indole-acetic acid and related acids are sufficiently soluble in water. Such salts, particularly the potassium salt, have been used and recommended by a few workers. Most of the alkali salts are not generally available.

The usual practice with the indole- and naphthyl-acetic acids as growth-substances is to rub them up with a little ethyl alcohol, in which they dissolve fairly readily, and to dilute that alcoholic solution with water to the desired

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volume. The presence of a small percentage of ethyl alcohol in the treatment solution is not harmful.

Other solvents have been tried by Tincker (1938).

Vapours

Vapours of "synthetics" do not appear to have been tried on cuttings, but see p. 54.

Additions or Reinforcements to Solutions

It seems that only one report has been made (Amlong and Naundorf (1938, C)) of the use of mixed solutions, containing, say, both indole- and naphthyl-acetic acids, and the effects of mixture do not appear to have been very marked, as compared with the effects of either component alone. Hubert *et al.* (1939; bottom of list B) found that a mixture of indole-acetic acid with ascorbic acid gave better results than the single substances. (See p. 68 and 80 for some work with ascorbic acid (vitamin C) alone applied to cuttings).

Evenari and Konis (1938, D) tried potassium permanganate alone and in conjunction with indole-acetic acid in solution, with various results, but the salt alone was not as effective in inducing rooting as was the acid alone. They found that sugar (sucrose; cane sugar) added to solutions of indole-acetic acid improved the rooting response, and they suggest that the addition of sugar to solutions of growth-substance may have considerable value.

Other Substances

The use of permanganate and of sugar as aids to root-formation is not new. A weak water solution of potassium or sodium permanganate is used by practical gardeners as a rooting medium for cuttings of some woody and semi-woody plants. Its rationale is obscure. Some suppose the permanganate to act by suppressing microbial growth, such as would occur if the cuttings were left long in unchanged plain water. It may conceivably produce growth-substances by oxidation of tissue-material of the cuttings, and it supplies potassium, which some writers overlook.

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The anti-microbial action of potassium permanganate received no support from the work of Curtis (1918), who has contributed the most extensive report of the action of the substance on the rooting of cuttings. His discussion of the rôle of permanganate may be commended to those interested.

Curtis also found that cane sugar in simple solution notably improved rooting of some woody cuttings. A favourable effect of glucose on the rooting of cut etiolated pea shoots has been reported by Amlong (1938, C). Cane sugar, given alone, was found by Evenari and Konis (1938, D) to stimulate callus formation in *Bignonia* and *Myrtus*.

Went (1934, A) used potassium permanganate (0.05 per cent. solution in water) to give "an effective disinfection" to etiolated pea seedlings from which the roots had been cut off in the course of preparation of the seedlings for a test of "rhizocaline, the root-forming substance". It is clear that Went did not impute to potassium permanganate any positive root-forming activity on such pea seedlings, and he wrote that the salt "decreases the number of roots on the controls". Webster and Robertson (1937) have reported, in a note, that application of potassium permanganate and related salts to some (presumably intact) plants (particularly the cactus *Opuntia leucotricha* growing in sand) produced a marked growth response (?) of the aerial parts), which the authors thought was not solely a manurial effect. No further details have been found.

Laurie (1928) found the following substances to hasten the appearance of roots on cuttings of the plants named, though they did not increase the ultimate percentage of rooting:

2 per cent. cane sugar in water: lilac, carnation, heliotrope, climbing Euonymus.

0.1 per cent. potassium permanganate in water: chrysanthemum and the above-mentioned four and some others.

Vinegar (three teaspoonfuls to a gallon (U.S.) of water): lilac, Euonymus, chrysanthemum.

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Potassium permanganate (one ounce to 8 gallons (U.S.) of water) was found superior to any "synthetic" tried, for root-production on kudzu cuttings. (Myers *et al.*, 1938).

The action of some of the minor elements, such as boron, zinc, and manganese, has been compared to that of vitamins. This is not the place for a discussion of the rôles of simple inorganic combinations of elements necessary for plant growth, but one organic compound has a special interest, as it has been claimed to be a succedaneum for inorganic iron. It has, apparently, no special effect in root-induction.

Iron is usually regarded as essential for plant growth, and it is supposed to have a special part in the formation of chlorophyll, although chlorophyll contains no iron. Pollacci and Oddo (1920) claimed that solution-cultures deprived of iron but supplied with other necessary elements and with magnesium 2-pyrrole-carboxylate, permitted normal growth of several species of plants. The pyrrole ring brings to mind the indole compounds and nicotinic acid; like inorganic iron, these may be growth-catalysts.

The work of Pollacci and Oddo was challenged by Deuber (1926) who found none but a toxic effect to be exerted by the pyrrole-carboxylate, even in minimal doses. That the substance had a stimulating effect was reaffirmed by Pollacci and Oddo (1932) for higher plants, and again by Pollacci for algae (*Ber. Deut. bot. Gesell.*, 1935, 53, 540).

Para-phenylenediamine has been successfully used by Hubert and Rappaport (1939) to stimulate root-formation on cuttings of several species of shrubs. It was used at a concentration of 100-1000 mg. per litre, at which strengths its action was reported to be very similar to that of the usual concentrations of indole-acetic acid.

Honey has recently been found by R. W. Oliver (*Sci. Agric.*, 1939, 19, 586) to exert a considerable stimulating action on the rooting of cuttings of *Thuja* and chrysanthemum.

CHAPTER VI

THE TREATMENT OF SEEDS

IN 1937 Grace suggested that if young lettuce, tomato, and other seedlings growing in sand or soil are treated with a solution containing indole-acetic acid or other synthetic growth-substance, a real stimulation is obtained with a dose of active substance at the rate of 50 to 250 mg. per acre (120 to 600 mg. per hectare). This is, so far, the ultimate in agricultural micro-therapy, and, should active substances be present in organic manures in only corresponding traces, their detection will provide a problem for the analyst. A pictorial height-curve of nasturtiums treated with such minute doses has been provided by Grace in two publications (1937, 1938 a).

Grace (1937) has also proposed two dusting techniques which could be used not only for cuttings but also for seeds.

An inert dust such as talc, or a standard mercurial dust disinfectant such as is used for cereals, had a small proportion of indole-acetic, indole-butyric, or α -naphthyl acetic acid mixed with it. Grace claimed that treatment with such dusts is safer than treatment with solutions. A toxic excess of growth-substance can be easily avoided in the solution treatment of such relatively massive material as cuttings. In the case of seeds, which are not only small but may be supposed while germinating to have little need of auxin-like material, it is very easy to give a toxic overdose of artificial growth-substance—as, indeed, the work of Grace has shown. Some toxic effects of solutions applied to seedlings are discussed in Chapter XI.

The dust technique appears to be peculiarly suitable for the treatment of seed with artificial growth-substances. Grace treated seeds of wheat, barley, and other plants, with a dust

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containing about 2-5 parts of growth-substance per million of dust, and grew them on in sand. Stimulation of the early stages of growth of the aerial parts was shown, and there was also a stimulated growth of the normal (underground) roots of wheat and barley in presence of concentrations of this order of magnitude.

Grace (1938 b) has shown that at least two synthetic growth-substances are compatible with, and stable in, formalin solution. A formalin treatment is widely used in Canada and elsewhere for the repression of that fungal condition in cereals known as smut. Smutted seeds treated by formalin have reduced germination (some of the seeds being dead) and the survivors have a weakened growth. According to Grace, such damage to seed oats and wheat can be considerably reduced, or entirely offset, by adding minute amounts of α -naphthyl or 3-indole acetic acid to the formalin solution before it is used on the seed. The strengths recommended were 0.01-1 part per million of solution. Such a mixture of formalin and growth-substance can be stored at least ten weeks. It is thought that the effect on the seed of the mixture of formalin and growth-substance should be attributed to stimulation of damaged embryos, rather than to prevention of damage at the time of treatment. It might seem that the artificial growth-substance replaces some natural hormone inactivated by the treatment, but there is evidence that the benefit cannot be so simply explained.

A demonstration of "the undoubted physiological activity" on winter wheat of mercurial dusts containing about 2-10 parts per million of the indole- and α -naphthyl-acetic acid was made by McRostie and others (1938). The dusts were applied to the seed, the comparison being between treatment with mercurial fungicide alone and fungicide plus growth-substances. Small but significant increases of yield of both grain and straw were reported, but the experiment is regarded as preliminary, and further work must be done to define the effects of variety, season, and other variables. This was the first field experiment with growth-substances applied to an agricultural crop.

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It may be noted that even should indole-acetic acid regain its one-time price of about 10s. (\$2·50) a gram, or about £200 (\$1,000) a pound¹, treatment of agricultural seeds on the field scale with a dust or formalin solution containing the recommended doses of synthetic growth-substance cannot add appreciably to the cost of dressing. Mercurial dusts, costing about 2s. a pound, are already applied free by many seed-merchants, and the added cost of material, if five parts of indole-acetic acid were incorporated, would not exceed a farthing (half a cent.) per pound of dust. It is, in fact, practicable to give away a very expensive synthetic chemical!

Work on the treatment of seeds with synthetic growth-substances has been done by Amlong and others (1937, 1938, 1939), who investigated mainly the effects of water-soluble potassium salts of indole-acetic, indole-butyric, and α -naphthyl acetic acids, in 10^{-3} and 10^{-2} equimolecular solutions to which had been added traces of magnesium nitrate and manganese chloride. 20 gm. of seed of sugar-beet (2 strains), lucerne, maize, and spring wheat were steeped 24 hours in 60 ml. of solution and sown by hand in the field. The results with maize (ears), wheat (grain) were not significant, but both strengths gave remarkable increases (24-62 per cent.) of yield of leaves and root of beet, and of lucerne hay. The authors conclude that the treatments are economically practical for these two crops. Seed treatment was also given to a number of herbs and vegetables grown in pots, and it was concluded that each species has its optimal dose-requirements.

Sprays of a commercial growth-substance preparation applied from four weeks after sowing gave especially remarkable results with sugar beet (about 60 per cent. increase in root and 80 to over 100 per cent. increase in tops). It was suggested that spraying has a specially beneficial influence upon the tops, and that the effect of seed-treatment is principally upon the

¹The cost is now slightly reduced; the prices of indole-butyric acid and the naphthyl-acetic acids are similar. There is reason to think that α -naphthyl-acetic acid should now cost shillings (a few dollars), rather than many pounds (hundreds of dollars) per pound. A cheapening of the other substances can scarcely be looked for.

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leaves. The spray treatment had little effect upon lucerne, however, and depth of rooting may be a factor.

In their 1938 paper Amlong *et al.* reported on the combined effect of two synthetics in mixed solution applied to seeds, and are probably the first workers to do so. The effect of mixture does not seem to be very marked.

The first investigation of the effect of a synthetic growth-substance on seeds was done by Cholodny (1936) in connection with the Russian work on vernalization (*syn. yarovization*), that is, the effect of applying physical treatments, such as cooling, to seed, for the purpose of hastening growth. Cholodny and others suspected a hormonal effect, and his so-called "hormonization" was an outcome, from which the whole of the other work on application of growth-substances to seed has probably derived.

The term "hormonization" is a vague one, because it can connote treatment with at least three classes of substance: (1) hormones proper to the species tested; (2) hormones belonging to another species of plant; (3) animal hormones. It has been used wrongfully to mean (4) treatment of seeds with synthetic growth-substances, and was in fact used by Cholodny in both senses (2) and (4) in his 1936 communication, wherein he described the effect of maize embryos on seedling growth of oats. (See also p. 39).

The treatment of seeds with urine containing animal hormones has been revived by Tovarnitzky and Statkovskaia (1938), who used urine as a source of sexual hormone; *cf.* Eyster and Ellis (1924).

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CHAPTER VII

The Use of Synthetic Growth-Substances in Grafting, in Producing Bud-Inhibition, and Other Operations

When a tree is grafted into another at the time of a certain conjunction of sun and moon, and is fumigated with certain substances whilst a formula is uttered, that tree will produce a thing that will be found exceedingly useful. . . .—MOSES MAIMONIDES, *The Guide of the Perplexed* (trans. M. Friedlander) as quoted by S. Tolkowsky, *Hesperides* (London: John Bale, Sons & Curnow, 1938).

Grafting ; Rooting of Grafted Woody Cuttings

IN view of the earliness of the demonstration (e.g., Laibach (1935, A)) that a synthetic substance such as indole-acetic acid applied in a lanolin paste may hasten or improve the formation of wound-callus, it is rather surprising that so few investigations on the application of the artificial growth-substances to practical grafting have been published. So late as the spring of 1939, a discussion of the possibility of grafting olives on to an Abyssinian stock plant was made, in which there was nothing but surmise concerning the mutual effects of the natural hormones of stock and scion and of an artificial growth-substance, if one were used (Tallarico (1939)). It is not, of course, to be expected that in the present state of plant-physiological knowledge any sound predictions regards such mutual effects could be made for any plant; still less could they be made for this almost unknown stock. The reason for mentioning this discussion is that it quoted no previous work, in spite of the fact that its author came from Rome, where the library facilities are excellent. One would suppose *a priori* that the lanolin smear method would be peculiarly suitable for grafting operations, though this suitability has been recently denied.

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A number of trials of indolyl-acetic acid as grafting reagent and some other new applications are recorded in the papers of Evenari (Schwarz) and Konis (1938). As these were published in a journal that is somewhat difficultly accessible, it may be useful to give here a rather full abstract, though it is not possible to mention every question touched upon in the papers.

These workers described, in their first paper, experiments with figs, olive, *Rosaceae* (apple, cherry, pear), and vine; and in their second paper, with several less important species (*Hibiscus*, *Ligustrum*, *Myrtus*, and some ornamental conifers). They used leafless (Part I) and leafed (Part II) cuttings, and applied the growth-substance in three modes: solution, lanolin paste and as actual crystals. They appear to be the first investigators to report the placing of crystalline growth-substance directly in contact with plant tissues.

These workers report with figs a number of findings regarding the relations between rooting and the possession of terminal buds by cuttings, untreated, and treated apically and basally. Indole-acetic acid applied to olive cuttings led to precocious shedding of leaves; in olive and *Rosaceae*, marked formation of callus was induced, but little root-formation. An apple grafting was improved by treatment, but in a pear and a cherry grafting the union of stock and scion was hampered by treatment.

On all varieties of *Vitis vinifera* tested, treatment improved rooting all round, though one mode of application was sometimes more effective than another, depending on the variety. In all varieties the union of stock and scion (so-called "English graft") was markedly improved by treatment, and in 1938 vines were grafted on a large scale at Ein-Harod with an indole-acetic acid treatment. The mode of treatment with growth-substances that was adopted in these two cases is not stated.

Traub (1938, B) has investigated the transport of natural hormones over the graft union of citron on sweet orange (the former being known to root readily, the latter with difficulty), and concluded that Went's factor for callus formation was transmitted, but not the factor for rooting. The cuttings

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formed no roots after being planted in a rooting medium. No artificial growth-substance was used, and it is not excluded that a synthetic substance might be useful if it were desired to root grafted cuttings.

Perhaps the most cogent paper that has appeared so far on the application of artificial growth-substances to grafting is that of Lefèvre (1939). He worked with vines (*Vitis vinifera*): scions of Pinot d'Ay on a strain of Berlandieri. He used four methods of application: two modes of spraying, immersion of the whole graft in a solution for 48 hours, and dipping the cut ends, only, for 48 hours. This last procedure seemed to be the best, both as regards production of roots and callus-formation of the union. He tentatively recommends a solution of 10 parts of growth-substance per 100,000 of water, and he thinks that for use on grafts indole-acetic acid may be superior to indole-butyric acid.

The review by Chouard (1939) should be consulted.

Inhibition and Stimulation of Budding and Flowering. Forcing

This is a development likely to have considerable importance. There is reason to suppose that much work of a mainly practical tendency is being done to extend and perfect the control of dormancy, and also that the results are being to some extent regarded as trade secrets, so that but little is being published.

The existence, in terminal buds, of a hormone (auxin) or hormones, affects the growth of lateral shoots, as has been shown by Thimann and Skoog (1933, F) and others. From this knowledge it is not a long step to consideration of an endeavour to control bud-development and flowering by the application of artificial growth-substances. If such control were achieved, it would be an important gain in several fields of horticulture: for example, for retarding flowering, and thus loss of blossom and of fruit, in fruit-growing districts liable to late frosts; and to the plant exhibitor, who could more accurately time the optimal development of his show material.

Though it does not directly concern the subject of syn-

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thetic growth-substances, Chailakhian's hormonal theory (1937) of flowering may be mentioned. This worker postulated the existence of a factor for flowering, which he named *florigen*. The mobility, and therefore the existence, of such a hormone was demonstrated in several ways; for example, grafts of *Perilla* on *Perilla* at different stages of flower-ripeness were made, and the grafts behaved as if the factor passed through the unions (*cf.* the grafting experiment of Traub mentioned on p. 52).¹ Practical applications of hormone theory in horticultural practice are explained in the 1937 paper, which has some 300 references. By appropriate techniques of vernalization (*see below*) transplanting, grafting, and by taking advantage of photoperiodism, the concentration of the flowering hormone can be controlled, and flowering may thus be either forced or retarded.

The *vapour* of methyl α -naphthalene-acetate has been found by Guthrie (1939) to inhibit the growth of buds of potato tubers. The substance is sufficiently volatile at a warm room temperature (25° C.) to produce its effects. The sprouting of whole tubers can be retarded by merely storing them at that temperature in presence of paper impregnated with the ester, *e.g.* in a bag. Epinasty of tomato leaves is readily induced if a little of the substance is placed in a bell jar with the plant.

Zimmerman *et al.* (1939) discuss "Responses of plants to growth substances applied as solutions and as vapours." There are similarities between the effects of unsaturated hydrocarbon gases (ethylene, etc.; used for forcing) and those produced by vapours of a number of "synthetics".

Gustafson (1936) seems to have been the pioneer in showing that known chemicals could cause the ovary of a flower to develop into a fruit. By using indole-butyric acid he obtained almost normal, but seedless, fruits from unfertilized tomato flowers. A good set of (parthenocarpic) fruit was secured by Gardner and Marth (1937-8) with holly and

¹In another paper Chailakhian and Zhdanova (1938) concluded that the natural growth-hormones affect growth only, and do not supply the stimulus for the formation of buds and flowers.

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strawberry by the use of synthetic growth-substances, the results with holly being thought to be of practical value to the florist. See also Nixon and Gardner (1939).

In a preliminary report, Bennett and Skoog (1938) give the results of applying yeast extracts and solutions of vitamin B₁, indole-acetic acid, and other substances to the cut tips of disbudded fruit trees kept in a warm greenhouse. The point about the greenhouse being warm is that cold is normally essential for the breaking of dormancy of fruit trees, just as subjection to a low temperature is an essential feature of the process of vernalization, which is used to "break dormancy" (induce earlier growth) in such seeds as those of winter wheat.

In these experiments of Bennett and Skoog the effect of the applied substances was not uniformly well marked. Vitamin B₁, while not notably influencing the number of buds produced, appeared to induce strong growth of such buds as did develop. The best effect was obtained by "injection" of extracts of autolyzed yeasts (brewers' and bakers'), especially in young Bartlett pear trees.

The following is a translation of the summary of a paper by Amlong and Naundorf (1938), after *Horticultural Abstracts*:¹

"Coating the dormant buds of lilac (variety, Charles X) once a day for seven days in succession with a hundredth- or thousandth-normal solution of indole-acetic acid, or with a thousandth-normal solution of α -naphthyl-acetic acid, or with certain other stimulating mixtures, results in a considerably earlier bloom. Covering the terminal buds with growth-substance pastes has a similar effect. The forcing effect of a hot-water bath can be increased by spraying the treated plant with indole-acetic acid solution once a day for a week."²

¹B.C.A. quotes what is apparently the same paper but gives elder as the experimental plant.

²A "normal solution", sometimes written *N* solution, is a chemical term here denoting the molecular weight, in grams, of a growth-substance theoretically dissolved in a litre of water. The molecular weight of indole-acetic acid is about 170; that of the naphthyl acids and the other indole acids which are used as growth-substances is rather more. There is, however, some permissible latitude in the strength of solutions, and for horticultural purposes no harm will be done if the molecular weight of all these substances is taken as 200. A hundredth-normal solution of indole-acetic acid would be prohibitively expensive, as it would contain (nearly) two grams in about a

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The mainly physiological work of Stoughton and Plant (1938) on sea-kale (*Grambe maritima*) should be consulted by those interested.

Warne (1937) has used sprays of indole-acetic and of α -naphthyl-acetic acids in 0·05 per cent. water solution to induce curling of chrysanthemum petals.

In a study which principally aimed at elucidating some physiological problems, Ferman (1938) suggested that it is not necessary to assume the existence of a hormone newly postulated by Went (1938) under the name *caulocaline*, and supposed to be a factor in the elongation of stems and lateral buds. Ferman described a number of experiments with solutions and pastes of indole-acetic acid applied to seedlings and cuttings, giving varying results. An aqueous solution of one part in 100,000 applied to the cut surface of a decapitated seedling of *Lupinus albus* inhibited the development of axillary buds, but more dilute solutions of that substance rather promoted their development.

For a report of trials with phenyl-acetic and indole-acetic acids (mostly as sprays) to attempt to bring about the breaking of dormancy of strawberries during the season September-April, see Roodenburg and Tiddens (1938).

Dihydrofolliculin has been shown to have an action similar to that of hypothetical flowering hormones (florigen, etc.), which may be chemically related to that animal derivative (Chouard (1938)). Butenandt has also suggested that one sexual hormone may be common to the animal and plant kingdoms.

The effect of some synthetic growth-substances on the production of new roots on transplanted trees (young red oaks) has been studied by Tilford (1939). Indole-butyric acid was more effective than indole-acetic acid; the cut ends were wrapped in sphagnum moss soaked in the solution, or the roots were soaked in a solution.

quart of water; the thousandth-normal solution is still costly. This application would seem to be one for which α -naphthyl-acetic acid is well adapted, and for which it could be extensively applied if its price comes down to a reasonable figure (see p. 48).

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Possible Non-Biological Applications

Robbins and Jackson (1937) have recorded an effect of indole-acetic acid on elongation of dead stem-wall material (cotton thread and hemp cord). These textiles stretched more, under the influence of a weight, when treated with lanolin containing 0·2 per cent. of indole-acetic acid, than when treated with lanolin alone. Stewart (1938) attempted to repeat this work and also extended its scope. He found no conclusive evidence of an effect of indole-acetic acid on the extensibility of living or dead stem- and root-cell-walls. Artificial silk (called by him "regenerated cellulose"), however, was found by Stewart to have an increased extensibility in dilute solutions of certain organic acids, such as acetic and oxalic, which he says are "known not to be growth-substances",¹ as well as in 0·2 per cent. aqueous indole-acetic acid.

¹At the Chelsea Flower Show of 1939 an unknown interlocutor told the author that several years ago he had obtained excellent rooting of *Bougainvillea* cuttings in India by treating them with dilute formic acid; untreated cuttings failed almost wholly to root. It is not known to the author how this substance was introduced into use for that purpose; it may represent an echo of some work by H. E. and E. F. Armstrong (*Annals Bot.* (1911), **25**, 507; *Proc. Roy. Soc. Lond. B.* (1910), **82**, 588), who examined some effects of dilute acids on leaves, but did not report root-formation, or apply the substances to cuttings.

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CHAPTER VIII

WHAT IS A ROOT?

IN popular language the idea "root" implies fundamentality, but, although the rooted plants normally bear their roots as their lowermost parts, there are many plants that have no roots at all and manage quite well without them. The algae—whether microscopic (*e.g.* diatoms), or big (the seaweeds)—have no true roots, the whole plant living bathed in a nutrient solution.

There are numerous parasitic plants which utilize other plants' roots. Some, like the sandalwood tree of Southern India, grow apparently independently, but have no true roots of their own and are root-parasites deriving nourishment from the roots of plants of other species growing near.

Another kind of rootless parasite grows on the ordinary aerial stems of its host without necessarily having any contact with the ground; parasitic plants of this type rely indirectly on the roots of their hosts—indirectly, because a portion of the host's stem intervenes between parasite and host-roots.

A root may be defined as an absorptive organ having a characteristic structure and possessing a growing-point protected by a [root-]cap.

Roots need not be underground; witness the pendant aerial roots in Fig. 1 and the supporting or buttress-roots of the banyan tree and the stilt-plant (*Pandanus* sp.). The latter possesses large aerial roots upon which the cap is often distinctly visible to the naked eye.¹

A root cannot, therefore, be defined as a fundamental part of

¹Excellent pictures of the plant *Pandanus sanderae*, including a close-up view of the aerial root-cap, are given in the article by John A. Moore, in the *Missouri Botanical Garden Bulletin*, 1933, 21, No. 10.

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a plant, nor can it be defined in terms of its position in the plant. Even thorns may grow underground.

It is now fairly well known that true roots can be induced to form in unusual positions: the property of being able to induce the formation of aerial roots, and to cause cuttings to form roots, is possessed by artificial growth-substances. This has given rise to no small amount of confusion, regarding the effects of the substances on existing roots.

Many people have mistakenly assumed that an application of a synthetic growth-substance to the normal underground roots of common plants must be beneficial. To explain why this assumption is wrong would take me beyond the province of this book into the sphere of plant physiology, but it may be hinted that the induction of new roots on rootless parts of plants—cuttings or stems—is one thing, and the elongation or growth of normally formed roots is another. While a root cannot be defined by an appeal to its position, the effects of a chemical stimulant may and do differ considerably, according to the position and original function of the part of the plant to which the substance is applied.¹

Went (1938 D) has shown that it is possible to separate two phases in the action of indole-acetic acid on the formation of roots on cuttings. The first phase is tentatively identified with a redistribution of the hormone rhizocaline within the stem. This phase can be induced by a number of substances not active in root-formation proper. The second phase can be induced only by indole-acetic acid and similar substances; this phase may be an activation of the accumulated rhizocaline.

There is at present no warrant for supposing that the addition of a synthetic growth-substance to a manure, or its presence in a manure, must inevitably improve the growth of ordinary underground roots. It is tempting to suggest that part of the special value of organic manures lies in their content of, or ability to form, substances like indole-acetic acid. But, until more is known about the presence in organic

¹The formation of shoots on or near the tips of roots of *Pogonia ophioglossoides* has been recorded by Margery C. Carlson (*Bot. Gaz.*, 1938, 100, 215), but the effect of synthetic growth-substances on this process does not seem to have been examined.

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manures of such substances, and the part they play (if present) in organic manures, invocation of growth-substances in such manures can be little more than insecurely-based sales talk. Vitamin B₁ may be responsible for some of the growth-promoting effect of organic manures. (See p. 68).

Many workers have found that synthetic growth-substances, and even auxin, are actually toxic under certain conditions to normal roots growing in solution-cultures. Some workers, such as Grace, have produced methods of application that are claimed to minimize the toxic effect, or even to convert it into a real stimulation of normal roots in sand or soil.

Some of the reported toxic effects are discussed in Chapter XI. It is not possible to sum them up, and the reader is therefore left to form his own conclusions about the paradox. It may resolve itself as knowledge grows.

CHAPTER IX

GROWTH-SUBSTANCES FROM NATURAL SOURCES

Agricultural Researches Having a Possible Bearing on Growth-Substances in Soil and Manure

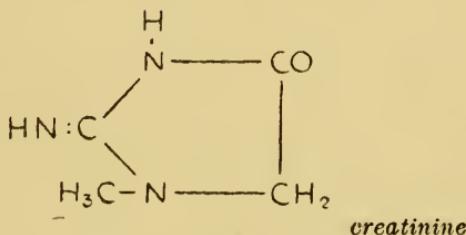
I have experienced, that the black water taken from a Dunghill, will make a *Cabbage*, or any of that Race, prosper extremely.—R. BRADLEY, *New Improvements of Planting and Gardening*, 1718.

THE action of mixed micro-organisms on proteins and tryptophan (3-indole- α -amino-propionic acid; indole-alanine) produces indole-acetic acid and other related growth-substances, as well as substances capable of acting as nutrient materials for plants. In nature and in agricultural practice, such substances reach the plant directly from the excretions of animals or via manure-heaps, in which they are mixed with decomposition-products of straw and other vegetable materials. Natural decay of leaves and roots, and the operation of composting, may also be supposed to yield growth-substances and related bodies. Rhizopin (indole-acetic acid) has, for example, been recognized as a product of a fungus, and fungi play an important part in the decay of plant-residues.

Recent work by plant physiologists and by botanists has tended to ignore the not inconsiderable mass of work done by botanists on the assimilation of amino-acids such as tyrosine, and by chemists on the existence of organic nitrogenous compounds in soil. The early work on amino-acids was almost all directed to proving their value as nutrients (sources of nitrogen) when the amino-acid was used as sole source of nitrogen. It therefore has only a comparative interest.

On the other hand, the work of the school of Schreiner and Shorey seems to bear with some directness on the question of the relation of protein-decomposition products and soil fertility. The studies of Schreiner and Shorey on nitrogenous compounds were more concerned with bodies related to the purine group than those of the indole group and phenyllic amino-acids. After their preparation of creatinine from soil, they broke new ground in two respects (Schreiner *et al.*, 1911): by trying the effect of creatinine on wheat-plants in water-cultures both with and without nitrate, and by demonstrating the presence of creatinine in stale extract of farmyard manure and in fresh cowpea vines.

Creatinine, and its relative creatine, which was also tested on wheat-plants, may have acted simply as nutrients. Creatinine is the anhydride of creatine, which is methyl-guanidino-acetic acid.



The experiments of Schreiner *et al.* were not fine enough for it to be possible to say that the increases of plant yield observed with creatinine and creatine in presence of small amounts of nitrate showed that creatinine, or creatine, is a growth-substance. The importance of the work of Schreiner and Shorey in the present connexion is rather that it focuses attention on the organic constituents of soils. As Schreiner and Brown wrote, in 1912, "the purely mineral-requirement theory of soil fertility has proven itself inadequate to cope with the accumulated facts."

A re-reading of the paper by Schreiner and Lathrop (1912) suggests in the light of present-day knowledge that decomposition products of protein (not necessarily of nucleo-proteins only) may be associated with the phenomena of "sickness" of

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highly-manured greenhouse soils, such as is treated by means of partial sterilization: growth-substances of the indole and other groups can be toxic in too great a concentration.

In a letter to *Nature* under the title "Rôle of Heteroauxones in Legume Nodule formation, Beneficial Host Effects of Nodules, and Soil Fertility", Link (1937) has suggested that "the beneficial effects of suitable concentrations of β -indole-acetic acid and other auxones may account in part for the characteristically beneficial effects of: (1) nodules for some host plants; (2) green manuring with nodule-bearing plants; (3) fertilizing with manures rich in dung and urine, or with compost; (4) humus soil; and (5) mycorrhizal fungi for some host plants."

This claim has been anticipated to some extent by other workers¹, and notably at some length in relation to green manures by Schreiner, Reed, and Skinner in 1907. Whereas unchanged tyrosine was found by Schreiner, Reed and Skinner (1907) to be toxic to young wheat seedlings, a tyrosine solution, after having become discoloured on standing, produced excellent growth of young wheat seedlings. The colour of this old tyrosine solution resembled that of manure extract, and the wheat plants grown in it resembled plants grown in manure extract. The darkening was possibly due to the oxidation of tyrosine to homogentisic acid (2:5-dihydroxyphenyl-acetic acid) which gave rise to further oxidation compounds of dark colour. The effect on plants of homogentisic acid has not otherwise been investigated. The chemical relationships of this substance have received attention owing to the occurrence of homogentisic acid in the urine of people subject to the rare condition known as "alcaptonuria" (see p. 74).

¹ In 1935 the author wrote: "One of the most remarkable effects of the leguminous crop, whether in mixture or in rotation, is its apparent ability to supplement animal manures. In peninsular Indian practice (the best-studied case) it would seem that legumes grown in mixture fill the place of animal manures. It appears unlikely that this ability is due solely to the nutrient nitrogen compounds supplied by the legumes, and it is probable that leguminous plants everywhere make a definitely biological contribution to the fertility of soil."—Hugh Nicol, "Mixed cropping in primitive agriculture," *Empire Journ. Exper. Agric.*, 1935, 3, 180.

Another ring compound investigated by Schreiner is picoline carboxylic acid (2-methyl-4-carboxy-pyridine). This was originally isolated from a virgin Hawaiian soil by Shorey (1907) and from other soils, and appears to have afforded the first instance of isolation and identification of a definite crystalline organic compound from soil. It was isolated from the virgin Hawaiian soil to the extent of 200 to 300 parts per million of soil—a not negligible amount. It was isolated from a Maryland soil by Schreiner and Shorey (1909) by a procedure fully described, though no quantitative details were given. The Maryland soil was "exceedingly" infertile, and Schreiner and Shorey were inclined to ascribe the infertility in part to the presence of the pyridine compound. On testing its effect on wheat seedlings, these authors found that in distilled water at a concentration of about one part per million "this soil constituent . . . is a stimulant, an effect which is, however, characteristic of small doses of poisons when applied to plants." Uvitonic acid (2-methyl-4: 6-dicarboxy-pyridine) showed no clear evidence of stimulation. It seems possible that a mono-carboxy-pyridine or similar compound may yet be found to have a real "growth-promoting" effect. The Maryland soil did not give notable yield-responses to application of either farmyard manure or artificial fertilizers. Whether this failure to respond was due to sufficiency of ring-compounds, or to excess of a toxic substance such as dihydroxy-stearic acid, must remain for the present an open question.

Thornton and Smith (1914-15) found that growth of the chlorophyll-bearing unicellular animal *Euglena* was greatly stimulated by small amounts of phenyl-alanine and tyrosine (hydroxy-phenyl-alanine; $\xrightarrow{\text{H}_2\text{O}}$ hydroxy-phenyl-acetic acid + NH_3), also by hay infusion; fresh hay infusion, or an infusion that has been long subject to decomposition by microbes, was appreciably less effective towards *Euglena* than an infusion subjected for a short time to the action of microbes. Tryptophan was not used in these experiments.

Bottomley (1915-17) suggested that the quantity of

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auximone (growth-substance) present in stable manure increased with the progressive decomposition of the manure, though here again a very well-rotted manure contained less auximone than did fresh manure.

Marked effects of dilute extracts and fractions of farmyard manure upon the extent of production of roots (in their normal positions in water-cultures) have been reported independently by Breazeale (1927) and by Niklewski (1931, 1935).

Breazeale used several dialyzed fractions of manure on wheat seedlings and concluded that the effect of the manure extract seemed to depend largely on the presence of black organic matter, and not upon the amount of plant-food ingredients it contained.¹ Minute quantities of peat-extract had a very striking effect on the growth of citrus seedlings deprived of organic matter but well supplied with nitrogen, phosphorus, and potash. Niklewski believed that the favourable effect of very dilute extracts of manure on the root-development of plants was due to colloids, but his earlier work was not chemically critical, as he apparently performed no dialysis on his manure extracts.

In a recent publication, Niklewski and Wojciechowski (1937) have brought forward a notable dossier of evidence that minute amounts of manure and peat extracts produce marked effects on the amount of growth of several kinds of plants grown in water, sand, and soil pot, cultures. Plants supplied with the organic extracts and complete mineral nutrients grew much more sturdily than did those supplied with complete minerals only. The extracts were not dialyzed. In the discussion there is, however, but little reference to colloids.

In a few preliminary field experiments, Niklewski and Wojciechowski attempted to see whether yield effects of normal doses (25 and 50 dz. per hectare) of compost applied as a late top-dressing to cereals had a relation to the water-soluble humus substances in the compost. Increases in yield

¹ Hitchcock and Zimmerman (1935, A) found that three indole acids could pass through a membrane impermeable to the dye Fast Green.

resulted, but it is not clear that these increases were significantly different from those produced by an equivalent amount of N-P-K.

The experiments of Hartley and Greenwood (1933) which showed marked increases in crops after application of the small dressings of 1 and 2 tons of farmyard manure, were for some time believed to show a special value of farmyard manure, since the equivalent amounts of nitrogen in the form of artificials failed to produce a similar effect. It was later shown, however (Hartley, 1937) that increases similar to those given by farmyard manure in these small dressings could be obtained by the use of equivalent amounts of readily-available phosphate.

The remarkably long-lasting yield responses to farmyard manure on one of the Hoosfield (Rothamsted) classical barley plots, still await a satisfactory explanation. Plot 7-1 annually received farmyard manure at the rate of 14 tons per acre from 1852 until 1871, but has been unmanured since. The yields of both straw and grain on this plot continue to be appreciably higher than those on the unmanured plots or on those receiving certain artificials without nitrogen. The average yield (since 1871) is about the same as that of the plot annually receiving 206 lbs. of sulphate of ammonia only, but is less than that of the plots receiving 275 lbs. of nitrate of soda.

In this connexion, the rather neglected work of von Liebenberg (1916) may be recalled. For a rotation manured once with a normal dose of farmyard manure and artificials, Liebenberg showed that doses of 20 and 50 kg. of farmyard manure per hectare, applied annually each autumn thereafter, produced appreciable, though small increases, in crops. Considerable tabular details of yields and composition were given. He wrote of the 50 kg. dose that nutrients as well as organic matter were active, but in the 20 kg. dose "dürfte wohl die organische Substanz allein oder in der Hauptsache die ertragsteigernde Wirkung verursachen."

By Mr. N. W. Barritt the author has been shown quince

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prunings, such as are usually discarded as valueless, which had developed well-marked roots after being "skewered" during March and April, 1937, into a compost heap formed largely of grass and turves.

Root-induction and cell-elongation are distinct. Zimmerman, Hitchcock, and Wilcoxon (1936) recorded that "so far, no growth-substances have been found to accelerate elongation in root-tissue. This peculiar response of roots raises the question whether there might exist in nature an entirely different type of hormone effecting elongation of the cells." Thornton and Nicol (1936) noted that elongation of one type of cell—the root-hairs of leguminous plants—was induced by secretions of legume nodule bacteria. Some properties of these secretions have been examined, but their chemistry is still obscure. Bottomley (1915-17) found that legume nodules were a potent source of auximones.

Vitamins

J. Bonner and Greene (1938) (with D. Bonner) have published figures of the vitamin B₁ content of three samples of cattle manure; they found 0.08-0.13 mg. of vitamin B₁ per kilo. of dung. A laboratory culture of Azotobacter, the free living nitrogen-fixing bacterium frequently present in fertile soils, contained over a thousand times as much. Since vitamin B₁ (thiamin, aneurin) seems to have a potently favourable effect on the growth of normal plant-roots, these findings may be important. The authors do not quote the pioneering work of Mockeridge (1920), but they say that the "long-disparaged 'auximones' of Bottomley" (1914, 1915-17) seem to rest upon a sounder basis than has been hitherto admitted.

V. G. Lilly and L. H. Leonian (*Science*, 1939, **89**, 292) have reported a demonstration of vitamin B₁ in soil.

Vitamin C was first used to accelerate root-production on cuttings (*Salix*, willow) by Davies, Atkins, and Hudson (1936), who found it to act similarly to indole-acetic acid, while less toxic. See also p. 43.

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CHAPTER X

SOME CONSTITUENTS OF URINE

Occurrence of Growth-Substances (other than the auxins and vitamins) in Urine

Urine, in a heat below that of boiling water, yields a clear liquor, of a very disagreeable smell, and which is found to contain nothing saline.—BEAUMÉ.

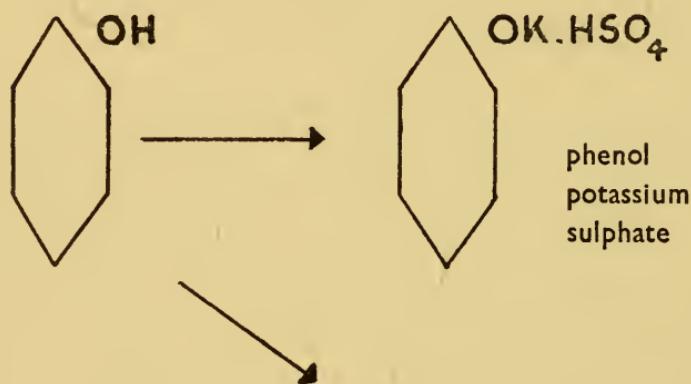
A LARGE number of substances able to affect growth and differentiation in plants are to be found in urine. Some of them (auxin-a, auxin-b) probably pass unchanged through the digestive tract, and, if they do, are integral plant-products. Others are fission-products of plant or animal protein, the latter being broken down either endogenously (in the body processes of the excreting animal) or exogenously (in the digestive tract, which is outside the body proper; or, less commonly perhaps, as a result of eating decomposed food, e.g., high game). Still another class is formed by those animal hormones which are capable of excretion in urine; of these the sex hormones are the chief.

In discussing urinary excretion, it should be borne in mind that to speak of urine as if it were one material, is often convenient, but does not always imply accuracy. The kind and amount of substances naturally present in urines, or excreted in urine after administration of a substance not a foodstuff, vary widely with the species, sex, age, and idiosyncrasy of the animal. Thus, phosphates are abundant in human urine, but are present in only small amounts in the urine of horned cattle; and hippuric and ornithuric acids, by their very names, remind us of special occurrences. The following outline should be read with such reservations, and

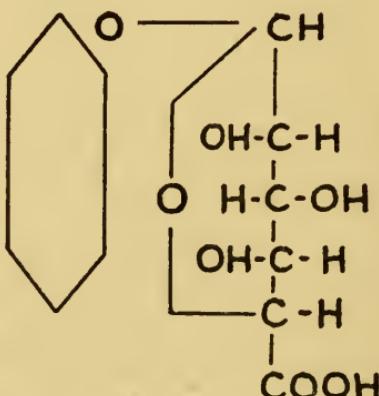
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with the further one that it is not intended as an introduction to urine chemistry in the wider sense.

Normally, benzene compounds possessing short side-chains are excreted as phenols. Free phenols sometimes exist in traces in urine, but phenol and cresols are usually found in urine as compounds of sulphuric acid and glucuronic acid. Such compounds are said by biochemists to be "conjugated". The combination (conjugation) prevents the phenols from attacking the walls of the urinary passage. It will be seen later that other irritant substances also have their caustic group "protected" or "detoxicated" by the formation of nearly neutral organic compounds.



phenol-
glucuronic
acid



Constituents of Urine

These phenolic compounds are not growth-substances. They represent the end-products of normal metabolism of such substances as tyrosine.

Phenylalanine, a constituent of almost all proteins, is oxidized in the body to phenyl-acetic acid, a growth-substance of considerable potency, and to phenyl-propionic acid. Phenyl-acetic and phenyl-propionic acids can also arise from the metabolism of tyrosine. When fed to animals, phenyl-acetic acid is said to form phenaceturic acid (a bland compound of phenyl-acetic acid and glycine) but human beings seem to be peculiar in employing glutamine as the detoxicating agent, forming phenacetyl-glutamine.

It is interesting to note that glutamine is used to conjugate phenyl-acetic acid only, and not its derivatives. Some of the urinary phenyl-acetic acid in human urine is combined with glucuronic acid.

Phenaceturic acid (phenylacetylglucine), being a compound of glycine with phenyl-acetic acid, is analogous to the better-known hippuric acid (benzoyl-glycine) a compound of glycine with benzoic acid. In fact the best natural source for the isolation of phenaceturic acid, as for hippuric acid, is horse urine, and both acids may be isolated from one sample of urine. It appears that the urine of herbivores is richer than that of carnivores in phenaceturic acid. The degraded sweet odour of horse urine is probably referable to its phenyl-acetic acid.

There seems to be a tendency for aromatic compounds having a fatty acid side-chain to be excreted in urine (in combination with glycine) as either benzoic or phenyl-acetic acid. The substances which, when fed, yielded benzoic acid, were those having an odd number of carbon atoms in the chain (phenyl-propionic, phenyl-valeric acids); while those acids having an even number of carbon atoms (phenyl-butyric, phenyl-caproic) appeared as phenyl-acetic acid. It will be noted that the formation of indole-acetic acid from tryptophan (indole- α -amino-propionic acid) upsets the beautiful regularity thus suggested.

The value of phenaceturic acid as a growth-regulating

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substance has not been tested, but it is easily hydrolyzed, and probably has the same value as its equivalent of phenyl-acetic acid. A few laboratory experiments have suggested that glycine, when used as *sole* source of nitrogen in the growing of plants, is fairly readily assimilable by plants, but such experiments bear little relation to practice. Some may be tempted to think that since phenaceturic acid comprises both a growth-substance and a source of nitrogen, it might be an especially valuable substance for plants, but it should be remembered that the amount of phenyl-acetic acid useful to the plant is probably much less than a molecular equivalent of the nitrogen required for growth. The growth-substance/nitrogen ratio in phenaceturic acid is 1, when expressed in molecules, but this is probably much too low for physiological balance; if the plant is to take up from the phenaceturic acid all the nitrogen it requires for growth, it will be left with a possibly toxic excess of phenyl-acetic acid. The point might be interesting to examine experimentally, but its nutritional implications do not seem likely to be useful. Phenaceturic acid, however, may find horticultural applications as a source of phenyl-acetic acid in cases where growth-regulation and not simple nutrition is the main object.

Hydroxy-phenyl-acetic acid in the form of *p*-hydroxy-phenaceturic acid has been reported in urine after administration of hydroxy-phenyl-ethylamine, but its importance under natural conditions is not known. It is almost certainly a growth substance. (See p. 65.)

Phenyl-propionic and β -*p*-hydroxy-phenyl-propionic acids also have been reported in urine. 2:5-dihydroxy-phenyl-acetic acid (homogentisic acid) occurs naturally in urine—in the free state, apparently, not conjugated—in persons subject to the rare condition known as "alcaptonuria". This is an inborn error of metabolism, owing to which the sufferers are unable to oxidize tyrosine and phenyl-alanine beyond the stage of the 1:2:5 compound. Alcaptonurics do not appear to suffer in health, but their urine blackens on exposure to air, owing to the rapid oxidation of the dihydroxy-benzene radical.

Constituents of Urine

Chemists may wonder how tyrosine (β -*p*-hydroxy-phenyl- α -amino-propionic acid) and phenyl-alanine (β -phenyl- α -amino-propionic acid) acquire the 2:5 hydroxy groups. It may be stated that proof of the physiological origin of 2:5-dihydroxy-phenyl-acetic acid from both tyrosine and phenyl-alanine rests upon the increased excretion of homogentisic acid after ingestion of either of the amino-compounds by an alcaptonuric.

Alcaptonuria is too rare a condition to have an agricultural importance, but consideration of it suggests that the black matter of manure- and compost-heaps may be in part a complex of oxidation products of dihydroxyphenols. For further information about the acid, see the section "Nachweis und Bestimmung der Homogentisinsäure im Harn", by W. Weise, in Abderhalden's *Handbuch* (1931), Abt. 4, Teil 5 (1), 551-560.

Coupling of a ring-acid with an aliphatic amino-acid (conjugation) seems to be of a fairly general occurrence. The methyl-pyridine compounds in 2-methylpyridine and picolinic acid, after administration by mouth, appears in the urine of dogs, rabbits, and frogs, as pyridinuric acid, a compound of picolinic acid and glycine, analogous to phenaceturic and hippuric acids.

Picolinic acid administered to birds becomes combined with the peculiar avian detoxicating amino-acid, ornithine, and is excreted as pyridinornithuric acid. Ornithine is normally found in combination with benzoic acid as ornithuric acid, but acetyl-ornithine has been reported as a plant constituent by Manske.

Indoxyl (hydroxy-indole) and skatoxyl are other phenols found in urine, of carnivora particularly. They are usually conjugated in the form of potassium sulphates; the indoxyl potassium salt is known as urine-indican. Their effect on plants is not known. Indole-propionic acid is stated to undergo hydroxylatón before excretion.

3-indole-acetic acid is the substance revealed by the old "urorosein" test (see Chapter XIV). Its properties and a brief account of its isolation from urine are given elsewhere.

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Creatine and creatinine, already mentioned on p. 50, are important and constant constituents of urine. They can almost certainly supply nitrogen to plants, at least after they have undergone bacterial decomposition. It is not known whether they can act as accessory growth-substances.

For much of the information given in this section, the author has relied upon the textbooks of biochemistry of H. H. Mitchell and T. S. Hamilton ("The Biochemistry of the Amino Acids": Chemical Catalog Co., New York, 1929, and of B. Harrow and C. P. Sherwin ("A Textbook of Biochemistry": W. B. Saunders Co., Philadelphia, 1935), and particularly in the latter the section on detoxication by A. M. Ambrose and C. P. Sherwin. See also the sections: "Nachweis und Bestimmung von Abkommlingen des Tryptophans im Harn", by W. Weise, in Abderhalden's *Handbuch*, 1931, Abt. 4, Teil. 5(1), 765-784, and "Von Tryptophan ableitbare biochemisch wichtige Verbindungen," by A. Ellinger in the same *Handbuch*, Abt. 1, Teil 7, 779-806, especially for E. and H. Salkowski's method of preparation from decomposed protein material.

To these and to similar sources the reader is referred for references and more details.



CHAPTER XI

CHEMISTRY IN RELATION TO GROWTH

Some Notions on Concentrations

Inhibition and Stimulation

THE growth-substances can act in extremely high dilutions. By the *Avena* test, auxin-a has been shown to be active at a concentration of 1 part in 110,000,000 of water; though ethylene in some circumstances is from 100 to 600 times more potent for equivalent molecular weights (Crocker, Hitchcock and Zimmerman (1935), as corrected by Hitchcock and Zimmerman (1935, A)).

Acute disturbances not unlike the effects of poisoning can be produced by the synthetic growth-substances in quite small doses; they are especially pronounced with young seedlings, grown in solution-cultures, and the question has on that account been asked whether it is not misuse of a term to call the synthetics growth-*promoting* substances (Leonian and Lilly, 1937). It is true that the substances do not always promote growth, even in large plants (Hitchcock and Zimmerman, 1935, A; Pearse, 1936-7A,) though they may initiate or control it. The doubt evoked by the toxic phenomena shown by seedlings is probably based on a misunderstanding of results of an unrealized overdosage.

This contention has received support from the work of Macht and Grumbein (1937) who have considered the question of toxicity of indole-acetic and indole-butyric acids and a naphthalene-acetic acid, from the point of view of the so-called diphasic biological effect familiar to toxicologists and pharmacologists. Their work is discussed in more detail below. In the present connexion it will suffice to quote a few words from their paper:

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"In the experiments reported by other writers (with the exception of Amlong [1936]) only the toxic phase of the drug action was recorded because of the concentrated solutions employed and the longer periods in which such plants were exposed to their action."

A fair size for the diameter of a plant cell is 0.02 mm. Supposing the cell to be a cube of 0.02 mm. side, a seedling of total volume of 0.8 ml. will have 100,000,000 cells. If all the indole-acetic acid contained in 1.0 ml. of a solution of 0.1 gm. of the acid per litre is uniformly absorbed by that seedling, each cell will contain 3.5 thousand million molecules of active substance. The number is roughly one-fiftieth of the number of molecules in a volume of air at N.T.P. equal to the volume of a cell.

Caution in dosage is still more necessary in working with excised root-tips having an initial total volume of the order of 1 cubic millimetre. As the excised tips are usually surrounded with many thousand times their weight of solution, the weight of active substance in the solution will have to be infinitesimal if harm is not to result.

Kögl *et al.* (1934) have shown that auxin-a can exert an inhibition of root-elongation in young oat seedlings. The effect was perceptible when tap water bathing the roots contained 0.01 mg. of auxin per litre. Indole-acetic acid was found to exert a similar, but less powerful, inhibition.

Marmer (1937) has made observations on the inhibition of primary root-elongation in wheat seedlings, testing the three homologous acids, indole-acetic, -propionic, and -butyric, with the refinement of working at two hydrogen-ion concentrations in phosphate-buffered solutions. At pH 4.6 the concentrations causing a 50 per cent. reduction in root-growth were respectively 0.012, 0.250, and 0.055 mg. per litre, while at pH 7.5, the same results required respectively 17.58, 4.57, and 0.56 mg. per litre.

The magnitudes of such figures must depend very much upon the conditions of experiment.

The apparent paradox that synthetic growth-substances, capable of promoting growth of apical cells of seedlings, and

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of causing initiation of adventitious root growth, inhibit elongation of true roots, has been studied by Amlong (1936; his paper has not been seen by the author) and by Macht and Grumbein (1937). The latter workers studied a new variable—the time of exposure of roots (of *Lupinus albus*; white lupin) to low concentrations of indole-acetic and indolebutyric acids, and an unspecified naphthalene-acetic acid. Concentrations as low as one part of acid in 5,000,000,000 of water or salt solution (*pH* about 5) were tried. Instead of a toxic effect, a small stimulation of root-growth was observed 24 hours after transferring the plants from growth-substance solutions in which their roots had been dipped for 15-20 minutes; an exposure twice as long usually produced a small inhibition of root-growth. No estimate of error is given, however, and it is not clear whether the plants not receiving growth-substance were subjected to a control operation of transference.

Hare and Kersten (1937) have appealed to a final absence of toxicity, towards plant roots, of dilute aqueous solutions of indole-propionic acid irradiated with ultra-violet light, as evidence of the disappearance of the substance. (They give reasons for thinking that methyl anthranilate was formed).

Unfortunately, none of the published values for minimal doses of solutions affecting root-elongation is comparable with the values for minimal doses able to produce cell-elongation by the oat coleoptile test; this is true even when oats were used for determination of both types of activity. The minimum dose producing bending of the coleoptile in the usual *Avena* coleoptile test is determined from results of tests on single plants, each plant being supplied with a minute dose of substance, of which it absorbs the whole or the greater part. On the other hand, tests of the toxicity of growth-substances towards roots have been made with batches of plants having their roots bathed in relatively massive amounts of solution, and the figures for the limiting doses that inhibit root-elongation to a prescribed degree have not been furnished as a dose absorbed per plant, as must be done for comparability with the coleoptile test. In reported results of the oat

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coleoptile test, limiting doses have been expressed as milligrams per plant (or what is in practice the same thing, a prescribed degree of bending of so many plants per milligram or gram of growth-substance), while the limiting values for root-inhibition have been recorded as milligrams per litre of solution, with either the number of plants or the volume of solution left unspecified.

Grace (1937, C) has made an attempt to solve the paradox of growth-inhibition caused by the synthetic growth-substances. He concluded that inhibition of growth was usually due to overdosage. His colleagues Newton and Jack found that as little as 1 part of α -naphthyl-acetic acid in 200,000,000 parts of water depressed the growth of wheat roots in length, though not in weight. Grace's dust treatment of seed (see page 46) apparently gets round the paradox by slowly releasing the active agent in amounts so small as to be stimulatory.

An excellent review of the subject has been presented by Borgström (1939) who has performed numerous experiments in an attempt to resolve the paradox. He used genetically uniform material (*Allium*) to determine the limits of concentration of various substances for the retardation or promotion of growth. Inhibition at relatively high concentrations was succeeded by a slight promotion at very dilute ones.¹ Like most other workers in this field, Borgström worked with roots attached to the plant, but he has carefully reviewed the possible interactions between applied synthetics and the plant's own hormones.

Borgström has also reviewed phytological effects of the vitamins ascorbic acid (C) and thiamin (B₁). The effects of these substances on roots is not restricted to a narrow range of concentrations. He suggests, therefore, that they may have a practical application, as they are "more convenient and far safer to use as actually growth-promoting chemicals for roots".

¹On page 79 the author has pointed out that there is no comparability between results of tests on roots and shoots, just because there is no common unit. Borgström has made a slip in thinking that the author brought this contrast forward as an *explanation* of the paradox discussed in this chapter. The author had no other intention than to make a plea to future workers to use consistent, or at least comparable, modes of stating their results.

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These two vitamins, according to Borgström, really accelerate growth of roots, and therefore are growth-promoting, in the sense used in this book.

Further evidence of a favourable action of vitamin B₁ has been provided by Bonner and Greene (1938, E) whose conclusions are interesting (see p. 68).

It may be suggested here that the glycerides of acids active as growth-regulators might be safe vehicles of application. They resemble the true fats in being non-volatile and in being practically insoluble in water, but can hydrolyze slowly. Methods of preparation of some lower phenyl glycerides are given by Darzens (1937).

Plant-Growth Substances in Tissue Culture

Some very interesting work has been briefly reported by LaRue (1935). He cultivated minute pieces of the hypocotyl (first shoot) of dandelion, wild lettuce, and ox-eye daisy. A mineral culture solution was used, one lot receiving indole-acetic acid in the very high dilution of one part in 20,000,000. Successful growth took place only in the culture receiving the indole-acetic acid. The resulting plants developed numerous leaves and extensive root systems, and were believed by LaRue to be the first successful tissue cultures in which complete plants had been grown. See also the paper of Gautheret (1937), who found that indole-acetic acid in appropriate concentration was favourable to the growth of cambial tissue of willow.

Kaufmann (1938) removed the cotyledons of seeds, let the seeds swell for two days in water, then placed them in the dark in media containing potassium salts, sugar (sucrose),¹ and indole-acetic acid. A 24-hour treatment with concentrations of one-millionth and one-hundred thousand millionth per cent. of growth-substance induced maximal growth of root.

Indole-acetic acid added to nutrient agar in which excised

¹This obligatory use of sugar in cultivation of excised roots, for which it replaces the normal photosynthetized sugar of whole plants, should not be confused with the use of sugar mentioned on pages 43 and 44. For other work on excised roots see p. 115.

Plant Growth-Substances

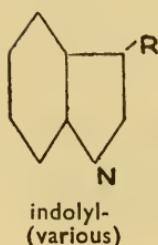
coleoptiles were grown retarded their growth in length (Avery and LaRue (1938)).

Duhamet (1939) has shown that indole-acetic acid inhibits or accelerates the growth of isolated (excised) roots according to dose, and has brought forward the fact that the substance acts not only on cellular growth, but also on the proliferation of root meristem. This he claims as new.

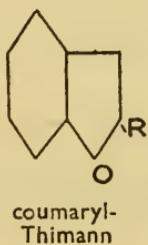
The effectiveness of salts, of esters, and of a nitrile as growth-substances shows that the favourable action of the synthetic growth-substances in proper concentration is not due to acid irritation, as has been pretended by a Continental school of plant physiologists. Quantitatively at least, the action of the natural and synthetic plant-growth substances accords with that of vitamins and hormones in animals. Very small quantities of either of the latter suffice to exert an optimal action upon the animal organism. The quantitative similarity is strengthened if the animal doses are calculated per gram instead of per kilogram of body-weight as usual. The animal is to some extent protected against overdosage of vitamins by the necessity of ingesting them through the gut. The plant itself has no obvious protection against excess of auximones offered to it in water solution, but it is possible that the colloids of manure and of soil are the plant's defence in this respect.

Chemical Constitution and Phytological Activity

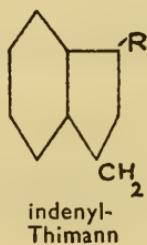
The following compounds (three of which are heterocyclic) have been shown to possess varying degrees of phytological activity, when provided with an acetic-acid side-chain. ($R = -CH_2COOH$). The names under the formulæ are



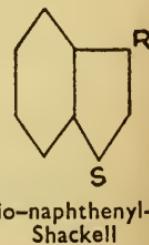
indolyl-
(various)



coumaryl-
Thimann



indenyl-
Thimann



"thio-naphthenyl"-
Shackell

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those of workers who have tested the action of the derivatives on plants, and are not necessarily those of the synthetizers. Thimann's coumaryl-acetic acid was called the 1-acid by him, as he excepted the oxygen atom from numbering. It is substituted in the same position as is the better-known coumarilic acid, called coumaryl-2-carboxylic acid by Heilbron (1934).¹

Shackell (1937), who reported the preparation of ²thionaphthene-3-acetic acid (by whom, is not clear, but see also the paper by Crook and Davies (1937)) found that compound inactive in the *Avena* (oat) test of coleoptile bending, but active in fairly high dilution by Went's etiolated pea method (see p. 129).

Thimann's results are most succinctly given in the form of a table, which also brings out the typical difference in activities revealed by different methods of assay, thus suggesting how much caution should be brought to attempts to correlate chemical constitution with an unspecified "activity".

TABLE.—APPROXIMATE ACTIVITY EXPRESSED AS PERCENTAGES OF THAT OF INDOLE-3-ACETIC ACID. (Thimann (1935))

	<i>By Avena coleoptile curvature.</i>	<i>By Avena straight growth.</i>	<i>By Went's (1934, J) etiolated pea method (p. 129).</i>	<i>By inhibition of bud-formation on etiolated pea stems.</i>	<i>By root-formation on etiolated pea cuttings Went (1934 A).</i>
Indene-3-acetic acid ...	1	7	20	14	definite ³
Coumaryl-1(2)-acetic acid...	0	about 0.3	4	4	definite ³

¹ The opportunity has been taken of correcting some mistakes which were made in Part III of the article in the *Manufacturing Chemist* (August 1937) before the author had access to the original of Thimann's 1935 paper.

² True naphthenes are C_8H_{16} . Thionaphthene is analogous to indene and indole, but the name thio-indene is not, however, sanctioned by custom,

³ Dependent on mode of application, etc.

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Four other furane derivatives tested by Thimann were found to be completely inactive by the two *Avena* tests.

For synthesis, and effect on plants, of coumaryl-2(3?)-acetic acid, see V. Titoff, H. Müller, and T. Reichstein, *Helv. Chem. Acta*, 1937, 20, 883 (from *B.C.A.*).

A number of discrepant results have been reported about the "activity" or "inactivity" of substances, in relation to their effects on growth. Substances judged as "inactive" or only slightly active, by workers using one test, have been found highly active by later workers using a different test-plant and technique, and possibly evoking a different type of physiological effect on cell-growth, proliferation or differentiation.

For this reason, amongst others, it has not seemed worth while to list and classify the numerous substances of which the physiological activity has been tested on plants.

A comparison of three plant tests (oat-coleoptile, etiolated pea stem, and "cylinder") has been made by Haagen-Smit and Went (1935), using a large number of synthetic substances, mostly of the indolyl and phenyl types. No naphthalene or higher aryl compound was tested in this series. *Allo*-cinnamic acid, ethyl *iso*-propyl indole-acetate, and 5-methyl-indole-acetic acid were reported to be equally as effective as 3-indole-acetic acid in the (Went's) pea test. Examined by the other two tests, no substance tried was more than a fifth as effective as indole-acetic acid, and only indole-3-pyruvic acid, 1-methyl-indole-3-acetic acid, 5-methyl-indole-acetic acid, and *allo*-cinnamic acid reached that degree of effectiveness. Though so effective in the pea and cylinder tests, *allo*-cinnamic acid and 1-methyl-indole-acetic acid were only weakly effective in the *Avena* coleoptile test, in which they produced an "abnormal" type of bending. Ordinary cinnamic acid was inactive in all three tests.

These authors wrote: "That steric conditions play an important part in the activity of these substances was confirmed by the activity of *cis*-*o*-methoxy-cinnamic acid, whilst the *trans* compound was inactive. In the meantime, the substitution with the methoxy groups has greatly diminished the activity." *Cis*-*o*-methoxy-cinnamic acid, however, was

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inactive in the *Avena* coleoptile test. The effects of other substitutions were briefly discussed.

The differing activities of the artificial growth-substances has been referred to their different behaviour towards what plant physiologists call "polar transport" in the plant.

An inhibitory influence of the terminal bud of plants upon the lateral ones has been known for a long time: it is the basis for some operations of pruning. It has been shown by Thimann and Skoog (1933) and others that the effect is due to nothing else but the production of auxin by the terminal bud: auxin is able to prevent lateral buds from developing.

Van Overbeek (1938) has attempted to explain how this inhibition might work. Went (1938) found that phenyl-butyric acid has no activity in the *Avena* test, yet is able to inhibit bud development to a considerable degree. Van Overbeek made use of this property of phenyl-butyric acid in order to be able to distinguish between the effects of hormone (auxin) produced by the plant, and the effects of an artificial growth-substance (phenyl-butyric acid) applied externally. Whereas other workers have recorded amounts of auxin or the activities of natural or synthetic growth substances in *Avena* units and thus in terms of auxin itself, Van Overbeek employed the novel unit of "gammas of indole-acetic acid equivalent per 1,000 gm. of water contained in the plant". This is a step towards standardization in terms of a known and widely-accessible pure substance.

The reaction of dormant leafless cuttings of willow to lanolin pastes or water solutions of indole-butyric acid has been studied by Pearse (1938). The formation of roots was greatly stimulated. When the portion of the base that had been treated was removed, the effect of the treatment was eliminated. A second treatment applied to a truncated cutting shortly after the end of the first treatment (by a 24-hour immersion in growth-substance solution) induced root-formation. Pearse thought therefore that the indole-butyric acid itself was the active agent that promoted root formation, and was itself used up or chemically changed in the process. He also thought that it may be more effective to apply

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growth-substances to the apical end of dormant woody cuttings. (*Cf.* Cooper (1935, A)).

No general correlation between chemical structure and phytological activity can yet be postulated. A substance affecting growth in one species of plant may be inactive towards another species, and, even within the simple series of analogues given at the head of this section no attempt to correlate activity towards plants with the 3-substituted position can logically be made.

D. Bonner (1938) has suggested that "compounds possessing the essential molecular structure probably all have the same activity in the *pH*-dependent and stoichiometric growth reaction, and that the differences in their observed activities are due to differences in activities in secondary processes." The latter may perhaps refer to Went's biphasic phenomenon (p. 60).

By correcting for difference in *pK*, so that only equimolecular concentrations of the free acid were compared, he found that *cis*-cinnamic acid had the same activity in the pea test as has 3-indole-acetic acid, which, on this mode of calculation, is superior to phenyl-acetic acid. He claimed to find a correlation between the dissociation curves and the activities at different internal *pH* of *cis*-cinnamic and phenyl-acetic acids.

For preliminary work on the effect of *pH* on the number of roots of pea formed in indole-acetic acid solution (an "optimum" curve was established), see Hubert (1938).

A short review of the chemistry and actions of growth-substances, from the standpoint of plant physiology (hormonology) rather than from that of the efficacy of external applications, has been given by Thimann (1938).

A more detailed discussion from the chemical point of view has been given by Koepfli, Thimann and Went (1937-8).

Tincker (1936 *a*, A) has reported that *iso*-indolinone-3-acetic acid (synthesized by Professor F. M. Rowe of Leeds) was inactive towards plants. Indole has been reported as inactive towards plants, but according to Glover (1937), the activity of skatole in the oat-coleoptile test appears to have been of the same order as that of indole-acetic acid. His

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suggestion that skatole may act in the plant as a growth-promoting *hormone* fails to convince, for lack of evidence that skatole is elaborated by the plant.

The report by Snow (1937) that benzoyl oxide and peroxide, applied in lanolin, produced perceptible bending in dark-grown oat coleoptiles, further complicates the issue. The activity of these substances was, however, only about one four-hundredth of that of indole-acetic acid.

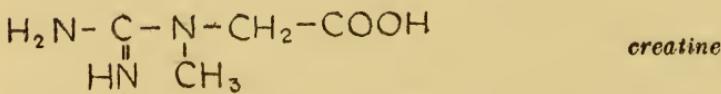
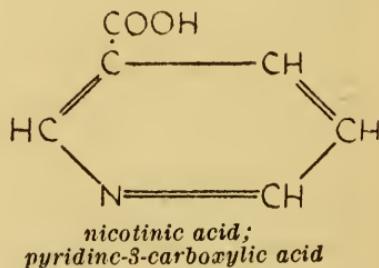
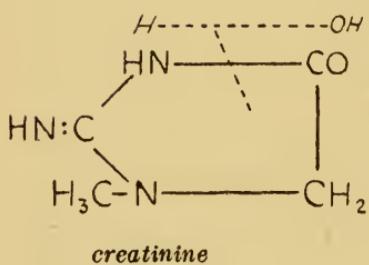
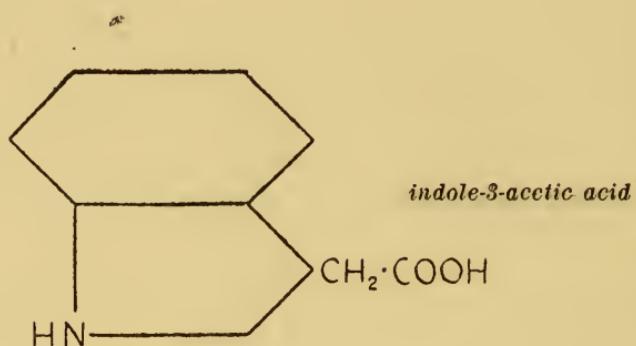
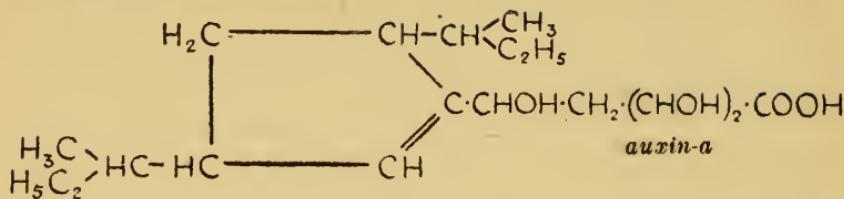
dl-indolyl-lactic acid was the only one of several indole compounds prepared by Baugess and Berg (1934) that was inactive as a growth-substance (Baugess, 1935). With one exception it appears to be the only substance capable of optical activity or resolution (other than *l*-tyrosine and *l*-tryptophan) of which the growth-regulating activity has been tested, hence nothing can be said at present about the relative growth-regulating power of most optical isomers.

The exceptional case which has been investigated is that of α - (β_1 indolyl)-propionic acid, synthetized by Kögl (1938) by a method not stated. This substance is optically active (its racemic form is probably the same as Ellinger's indole-methyl-acetic acid, p. 102 and 104). Kögl found that the *Avena* activities of the racemic (+), and (-) acids were, in 10^9 A.U. per gram, respectively 23, 48, and 1.6. Thus the (+) acid is about 30 times as active in the *Avena* test as the opposite enantiomorph is. See page 23 for reference.

Compounds related to tyrosine as well as those related to tryptophan have physiological activity. Extracts of thyroid gland (containing thyroxin, an iodo-derivative of tyrosine) were among the first of the animal hormones shown to affect growth in plants (Nicol, 1937). Hammett (1936) writing from a zoological standpoint, has suggested that attention given to the iodine in thyroxin has overshadowed the importance of its tyrosine residue.

As a matter of interest, and without comment (except to point out that creatinine and creatine have not been shown to be growth substances in the modern sense of an accessory rather than a nutrient), the formulae of auxin-a, indole-3-acetic acid, creatinine, and creatine may be given side by side:

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24, 135; see also Solacolu, T., and Constantinesco, D., *Compt. rend.*, (1936), 203, 437.

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CHAPTER XII

CLASSIFICATION AND NOMENCLATURE OF PLANT GROWTH-SUBSTANCES

Becher and Stahl, pursuing further and further the combinations of the primitive principles, and of those which they term secondary, establish different orders of compound bodies, to which they give improper denominations. The signification of many of these terms is even contrary to the ideas usually affixed to them, and is liable to occasion obscurity.—BEAUMÉ.

FROM at least a historical point of view, the three following growth-substances seem to form a class: auxin-a (auxentriolic acid); auxin-b (auxenolonic acid); and "heteroauxin" (3-indole-acetic acid). To these may be added the synthetic substances, homologous with or akin to indole-acetic acid, and principally interesting the readers of this book.

In addition to these, however, there is a bewildering variety of more or less well-characterized substances derived from animal glands, for at least some of which claims have been made that they are capable of affecting plant growth (see, for example, the paper by Havas and Caldwell, 1935). Amongst these may be mentioned the male and female hormones, the chemistry of which has reached a fairly advanced stage, owing largely to the work of Marrian and of Butenandt. For the chemistry and names of most of these substances, the books by Harrow and Sherwin (1934) and Bredereck (1936) should be consulted.

Another, but ill-defined, class of substances is that known chiefly for its effects on microbial growth. Amongst these may be mentioned Williams's (1933) "pantothenic" acid, so named on account of its wide occurrence in living material,

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and Allison and Hoover's (1933) "co-enzyme R", which can be obtained from cane sugar; also reported to be widely distributed.

One more class of substances needs to be mentioned as having growth-regulating properties towards plants, and that, strangely enough, is formed by the vitamins, especially vitamins C (ascorbic acid) and B₁ (thiamin).

This outline classification has been made partly for the sake of completeness, and partly to render intelligible the paragraph on nomenclature which follows: it is uneasy to discuss the action of any group of substances on plants unless we are sure which group we are discussing. No doubt the borderlines between the above-mentioned groups will shift as well as become clearer with increasing knowledge. As things are, it would be a pity to lump together in one comprehensive group such substances as: auxenolonic and ascorbic acids; the phenyl-, indolyl-, and naphthyl-acetic acids and esters and nitriles thereof; together with adrenalin and impure theelin: under some such name as "phytohormone", merely because they can all affect plant growth in some way. Cf. Eyster and Ellis (1924, C).

What a Hormone is¹

The term "hormone" is inapplicable to designate a class of substances which affect the plant's growth only after having been applied from without. The first hormone was discovered by Bayliss in 1902. It was a substance (not yet properly identified) secreted by a part of the intestine, and acting as a sort of release for digestive processes lower down. Previously, it was thought that body processes were controlled by telegraphic impulses along nerves, but Bayliss showed that some processes were controlled, not by intangible messages, but by actual substances. The word "hormone" was adopted to signify a chemical messenger. Whereas a painful sensation,

¹ Part of this section, as well as the introductory paragraphs of Chapter I, have been reproduced from the article "What is a plant-hormone?" (*Fertiliser, Feeding Stuffs, and Farm Supplies Journal* of 28th July, 1937, 22, 407) by kind permission of the Editor of the *Fertiliser*.

for example, is like an unpleasant telegram, a hormone can be likened to a key, a bundle of manuscripts, or a sample of merchandise—that is, *something* passing between two parties and essential to the carrying out of a transaction.

Note, however, that Bayliss's hormone, and the many others discovered since, all pass between two parts of the same body. A medicine or a poison administered from without may produce a very marked result on the body, but it is not therefore a hormone. Vitamins are not hormones, because they have to be supplied to the human or animal body from a plant source—that is, from outside.

Phenyl-acetic acid or its more powerful relatives, such as indolyl-acetic acid, produce remarkable effects on plants, particularly by way of initiating adventitious root-growth. They are, however, more like vitamins than they are like animal hormones, and it would be fair to call them vitamins-for-plants. In any case, it would be misleading to continue to call them hormones, *unless* it can be proved that they are formed within the plant and act as natural chemical messengers within it.

There can be no objection to an extension of the meaning of the word "hormone" to connote substances so acting in plants. There is a small class of growth-substances, which do occur in plants and do regulate growth within them. Vitamin C is produced in the plant and regulates some growth-processes within it (von Hausen, 1936). On the basis of von Hausen's work, vitamin C is a type of true plant-hormone, in which class come also the two auxins, the plant female hormone of Butenandt, and possibly a male hormone. On the other hand, such products as those of decomposition and of putrefaction, whether derived from processes taking place in the animal gut or in the compost heap, or derived from synthetic processes, are supplied externally to the plant and cannot be called hormones: they are auximones, or vitamins-for-plants. Unless it can be proved that the "synthetic" growth-substances occur naturally in plants, and regulate growth within them, it would to-day be as absurd to give the name "hormone" to these exterior substances which affect

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plant growth as it would be to confuse vitamins with hormones in speaking of animal physiology.

Link *et al.* (1937, H) have proposed a complicated nomenclature for all growth substances.

Their "autoauxone" is apparently an unnecessary synonym of "hormone" (*sensu stricto sive plantarum sive animalium*). A hormone is excellently characterized by the phrase of Link *et al.*: "produced by the individual whose development it affects." "Auxone" is much the same as Bottomley's "auximone", and not obviously preferable thereto. The additional term "heterauxone" (or possibly "heterauximone") brings to the nomenclature a little more definition than does "auximone" alone, but only if "auximone" is used in the most general sense to mean any accessory substance (not an ordinary nutrient) affecting growth and cell-differentiation. It appears that historical and linguistic propriety would be satisfied if the term "hormone" were reserved in its usual sense, and "auximone" (or "phytamin") were used for substances affecting growth of an individual not producing them.

Link *et al.* have performed a useful service in pointing out the need for distinguishing between different modes of tissue growth, but the necessary distinction can be made without resort to ponderous words to designate the incitants of such growth-modes.

An unexceptionable definition of a hormone is that given by Went and Thimann in their book (mentioned on p. 23). It is:

"A hormone is a substance which, being produced in any one part of the organism, is transferred to another part and there influences a specific physiological process."

This covers both animal and plant hormones. If it is desired to distinguish hormones of plants from those of animals, the former may be called *phytohormones*. This term is widely known, though comparatively seldom used in its proper sense; it is often used to connote a synthetic growth-substance, *i.e.*, a drug applied from outside. The acceptance of

phytohormone may ease the way for the word *phytamin*. The latter term has been tentatively accepted by Loehwing (1937) and has been substantively used in its French form *phytamine* (f.) by some Italian and French authors.

The definition of a hormone given by Went and Thimann excludes drugs, and is sound from a physiological point of view. It is to be hoped that other physiologists working with phytohormones or interested in the effect of synthetic drugs will call upon their physiological training so as to discriminate between what is a hormone and what is not.

The physiologists present at the First Phytohormone Conference, held in Paris in October 1937 under the chairmanship of Professor Boysen Jensen, apparently viewed the matter in a similar light. For their preliminary classification, see *Chronica Botanica* (1938), 4, 48.

Lefèvre (1939) has recorded the concentrations of 3-indolyl-acetic acid in fresh leaves and roots of various normal and pathological plants. If the natural occurrence of this substance in plants is accepted, it may be a true hormone.

Group Nomenclature for Plant Growth-substances

In 1934 the author suggested the name "phytamin" as a generic term for the accessory food factors of plants (vitamins-for-plants), for which numerous names and adjectives have been proposed (auxins, plant hormones, growth-regulating and growth-promoting substances, etc.). The name *phytamin* was intended to cover the sense of Bottomley's (1915-17, C) "auximone", of which Bottomley wrote: "This term may usefully serve as a general descriptive name for these organic plant growth-promoting substances until our knowledge of their true nature and composition is sufficiently extended to warrant the application of a more satisfactory name." The author proposed the term "phytamin" in substitution of "auximone", because Bottomley's work was generally discredited as a result of the exaggerated claims made for his commercial preparation, "bacterized peat".

Bottomley may have been led too far in his inferences, but it can now be suggested that his laboratory demonstrations

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of the growth-promoting value of extracts from rotted organic matter were sound.

In view of this, it seems desirable to restore the term "auximone", which is historically valid. It should be remembered that growth-regulation and growth-promoting are not synonymous. If "auximone" is thought not to be logical (Greek *auximos*: promoting growth) there would seem to be no alternative to the generic use of either "phytamin" or growth-regulating substance: the latter may, however, be shortened to growth-substance.

The term growth-substance, or growth-regulating substance in the current sense as applied to plants, was introduced by R. Snow about 1928.¹ It is admittedly not free from objection, being vague (Avery (1937)) but, if used in conjunction with the adjective "synthetic", expressed or understood, it at least avoids confusion with hormonal substances. These also are, of course, growth-substances or -factors, but, being hormones, such can be definitely named as a class. We thus have the division of all plant-growth substances into:

- (1) Hormones (phytohormones):
 - (a) auxins (auxin-a, auxin-b).
 - (b) vitamins (B₁, C, . . .)
 - (c) rhizocaline, florigen, . . .
- (2) Synthetic growth-substances (drugs).

This classification is not ideal, for it suffers from the classificatory disadvantage that vitamin C is a true hormone in the plant, but when applied as a drug it enters division (2), producing as a drug a possibly different effect from that which it brings about hormonally.

Division (2) might be divided in terms of Went's biphasic actions, but his name sub-auxin for e.g., phenyl-butyric acid, does not seem logical, as that substance is not an auxin or chemically auxin-like, and is not a hormone.

The term "auxin" for the whole class of growth-substances

¹The acknowledgment given to Dr. T. Eden in the first edition was meant to thank him for bringing this term to the author's notice—not that Eden has introduced the term into general use. It is regretted that this was not made clear.

Nomenclature

is undesirable so long as it is also used as a trivial name for the substances at present known as auxentriolic acid (auxin-a) and auxenolonic acid (auxin-b).

Williams (1928) has suggested the word "nutrilite" to mean any substance that affects the growth of organisms, but the need for such a broad term does not seem to have been generally felt.

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CHAPTER XIII

SYNTHESIS

(With notes on the auxins and vitamins)

Putrefaction may, I conceive, be considered as a spontaneous analysis, without heat; or a subsidence and laceration of the particles of bodies, by the weight of their mass, and by the dilatation of the fluids they contain, but aided by the external heat of the atmosphere. This spontaneous analysis disengages the aqueous, oily and saline principles of which the bodies consist.—BEAUMÉ.

IN company with many foul-smelling substances, there occur amongst the products of decomposition and putrefaction a number of ring compounds having a pleasant odour. Some of them, such as phenyl-acetic acid, have a use as perfumery synthetics, but their discovery in putrescent meat and the like was made long before any commercial application was found for them.

Indole-acetic acid has long been known as a product of natural decomposition of protein. Pathologists have devised a method of testing—the “urorosein” test—for its supposed presence in urine (see p. 123). A positive result from this test has been stated to be suggestive of intestinal disorder. There is little doubt that indole and indole-acetic acid in nature are derived from the breakdown of tryptophan contained in many proteins.

It has recently been found that many ring-substituted fatty acids possess remarkable powers of altering the modes of growth of plants. The first of these compounds to be chemically identified after its growth-regulating properties had been recognized was indole-acetic acid from urine. It was originally called “heteroauxin” by the plant physiologists. Indole-acetic acid occurs in urine much more frequently than the old uroro-

sein test indicated, and by the sensitive methods of plant physiology, it can be shown that by virtue of their content of "auxins", most urines are capable of affecting cell-growth in plants. Phenyl-acetic acid is also capable of affecting plant growth, as are a number of homologues.

Effect on Plant Growth

Looking back, it seems a little remarkable that more curiosity was not shown by agriculturists and plant physiologists about the effects on plant growth of the products of protein breakdown. From consideration of the universal esteem in which composts and animal manures have always been held, it might reasonably have been deduced that something was present in them that was not explainable in terms of N,P,K, and "humus". The fact is that elemental chemistry (with some quasi-mystical appeals to the unknown *humus*) has held this field. Investigation of the ability of certain products of protein breakdown to regulate growth in plants began without references to the origin of the substances; but when the composition of "heteroauxin" had been ascertained, that substance was found to be identical with the long-known decomposition-product, indole-acetic acid.

Already a considerable number of substances shown to possess growth-regulating properties in plants have been found to occur naturally, and many have been synthetized. A description of the chemistry alone of these substances would fill a small book, which would be out of date as soon as printed. Therefore, no attempt will be made here even to list the recorded growth-regulating substances, but an idea will be given of the synthetical preparation of some representatives of each complex group, excepting only the active phenyl compounds such as phenyl-acetic, phenyl-propionic, and *allo*-cinnamic acids. Interested chemists, having consulted the standard textbooks, will find more recent details in the substantially complete references at the end of this chapter. Being intended to introduce some physiological, as well as chemical, notions, the account is not presented in an entirely logical chemical order.

Plant Growth-Substances

Positioning and Nomenclature

The indole molecule consists of one five-membered (pyrrole) ring (composed of one NH and four CH groups) having two CH groups in common with a benzene ring. Numbering of substituents in the pyrrole ring usually commences with the N group as 1, proceeding around the pyrrole ring away from the benzene ring. The pyrrole nucleus sometimes bears the letters α and β in place of 2 and 3, substituents attached to the nitrogen atom then having the prefix N. The practice of using Greek letters to denote position in the indole nucleus is likely to lead to confusion, especially in considering compounds, like tryptophan (3-indole- α -amino-propionic acid), in which Greek letters also serve to indicate positions in a side-chain.¹ The prefix *Pr* has also been used to indicate substitution in the pyrrole ring. The use of this prefix, which does not affect the numbering as given above, is almost extinct.

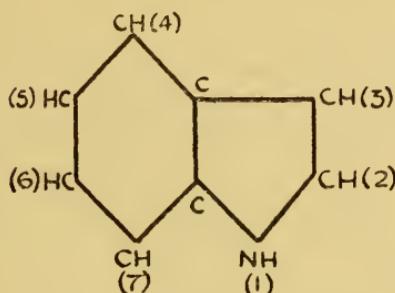
Most of the naturally occurring indole derivatives are substituted in the 3-(β) ring position. 2-substituted derivatives are perhaps less often synthetized than the 3-substituted, though 2-carboxy-3-methylindole (2-carboxy-skatole; isomeric with indole-3-acetic acid) was made by Wislicenus and Arnold as long ago as 1887. Recently, indole compounds substituted in the benzene ring have been synthetized and tested for activity upon plant growth. The prefix *bz-* is used to indicate substitution in the benzene nucleus, numbering then proceeding round the benzene ring consecutively with the numbering of the pyrrole ring. The atoms common to both rings are, as usual, not numbered.

The ring nomenclature of the indolyl and related compounds is not standardized. Sometimes the termination -ole is used, sometimes -olyl; in the case of relevant derivatives of naphthalene and other higher hydrocarbons, the tendency seems to be to retain the ring name unchanged. This confusion is unfortunate, but no greater degree of precision can be expected while the Chemical Society (Smith, 1936) write

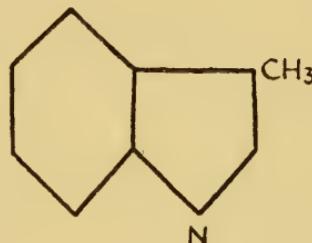
¹ The author has used the α - and β - notation in discussing the naphthalene compounds, instead of using the more modern 1- and 2-.

benzenesulphonic acid and phenyl-acetic acid, the simple C₆H₅ group having two names while fulfilling similar functions. The Chemical Society appear to prefer the form indole-acetic to indolylacetic, but, to the author, the latter seems desirable from the analogy of phenyl-acetic. Jackson and Manske (1935) have introduced the term indylene to indicate at least some di-substituted derivatives of indole, in analogy with phenylene.

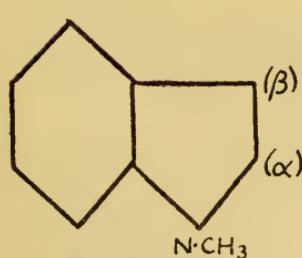
TYPICAL FORMULAE OF GROWTH-REGULATING AND ALLIED SUBSTANCES OF PHYSIOLOGICAL IMPORTANCE.



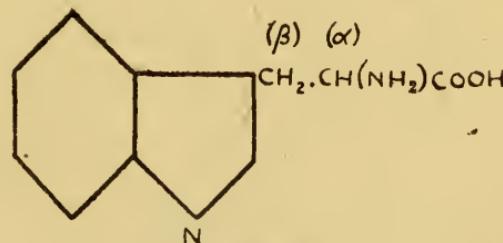
Indole;
benz-pyrrole.
C₉H₇: O₂H₂: NH



Skatole;
3-methyl-indole;
Pr-3-methyl-indole;
β-methyl-indole.



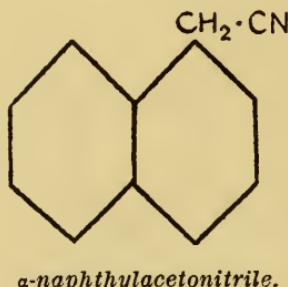
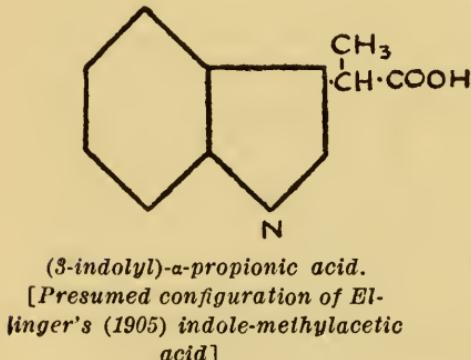
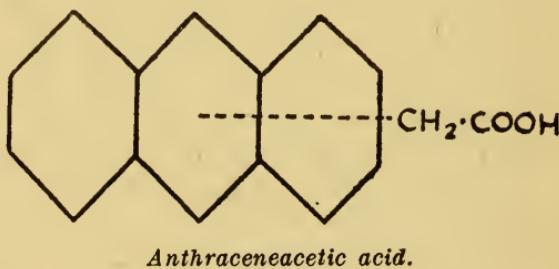
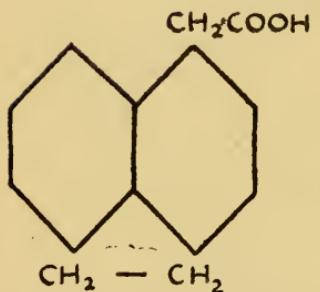
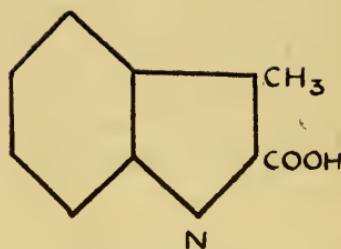
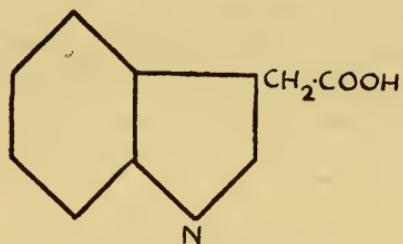
1-methyl-indole;
N-methyl-indole.

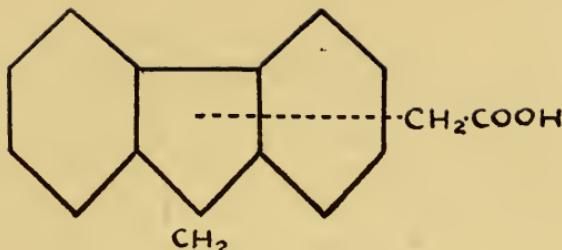


Tryptophan;
α-(3-indole)-amino-propionic acid
(optically active).

Plant Growth-Substances

TYPICAL FORMULAE contd.





Early Work on Indole Compounds

Indole-acetic and -propionic acids bear a close relationship to the tryptophan constituents of proteins. An excellent outline of the earlier researches on the constitution and synthesis of indole-acetic acid and its congeners is given in the article "Tryptophan," by Martha A. Whiteley, in Thorpe's Dictionary of Chemistry (1916 edition). Tryptophan is 3-indole- α -amino-propionic acid. It is somewhat surprising to learn that tryptophan, discussion of which now occupies a prominent place in many textbooks on biochemistry, was discovered so recently as 1901 (Hopkins and Cole), though the name dates from 1890. Skatole is 3-methyl-indole. A substance called skatole-acetic acid was isolated by Nencki in 1889. The synthesis of indole-3-acetic acid was not accomplished until Ellinger in 1904 performed it to follow up some problems of constitution raised by the work of Nencki and of Hopkins and Cole. Ellinger showed that it was identical with what Nencki had called skatole-carboxylic acid. In 1905 Ellinger further showed that the substance called skatole-acetic acid by Nencki was identical with indole-3-propionic acid. The preparation by Hopkins and Cole (1903) of indole-acetic acid from *l*-tryptophan by the agency of an anaerobic bacterium has now only a historical interest for chemists, but as it introduces the time factor, it retains an interest for those concerned with the biological production of growth-substances.

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First Syntheses

Emil Fischer's method of ring-closure was used by Ellinger (1904) for the first synthesis of indole-3-acetic acid. The phenylhydrazone of methyl aldehydo-propionic acid, on heating with zinc chloride, or, better, by prolonged heating with alcoholic sulphuric acid (Wislicenus), yielded methyl indole-3-acetate. The aldehydo-propionic acid was prepared



in fair yield by boiling aconic acid | | with water.
O.CO.CH₂

The aconic acid was prepared from commercial itaconic acid; for details and references, the original should be consulted. In his next paper, Ellinger (1905) described a preparation of the now usual indole-propionic (β -3-indole-propionic) acid by a tedious and probably uncommercial process, having γ -aldehydobutyric acid as intermediate. In the same paper Ellinger also described a similar preparation from aldehydo-*iso*-butyric acid of the compound isomeric in the side-chain (α -3-indole-propionic acid), for which he used the strange-sounding name indole-methylacetic acid.¹

Relation to Animal Physiology

It is of interest that several of the more recent preparations of indole acids—some of which are now known to have remarkable effects upon the growth of plant organs—had their genesis in a study of the rôle of tryptophan in animal diets. Jackson (1929) devised a diet deficient in tryptophan, in substitution for which various indole compounds were tried. Indole-pyruvic acid was the only one of these compounds capable of replacing tryptophan in rats' diet; it caused resumption of growth in animals deprived of tryptophan. A mixture of pyruvic acid and ammonium pyruvate leads to the formation (de Jong, 1900, 1904) of the type of amino acids most

¹ The name is correct provided that the methyl group has not wandered into either ring. It suggests an interesting chemical catch. Q. What is methyl-acetic acid? Ans. Propionic acid. Q. Is, then, indole-methyl-acetic acid identical (for any one point of substitution in the indole nucleus) with indole-propionic acid?—Try it on your friends!

common among protein derivatives—the α -amino acids; this suggests an explanation of the values of indole-pyruvic acid as a substitution for tryptophan.

The immediate chemical interest of Jackson's 1929 paper lies in its preliminary description of new or improved methods of preparation of several indole compounds, amongst them the following: indole-3-butyric acid from application of the Fischer ring-closure to the phenylhydrazone of ethyl hydrogen α -ketopimelate (full details of this synthesis were published by Jackson and Manske (1930)); indole-3-propionic acid was prepared according to Kalb, Schweizer, and Schimpf (1926); indole-3-pyruvic acid was prepared by the method of Gränacher, Gerö, and Schelling (1924).

Berg, Rose and Marvel (1929-30), who also were interested in the animal metabolism of tryptophan, reported the preparation of indole-3-propionic acid from adipic acid. Diethyl adipate was prepared and condensed with itself (van Rysselberge, 1926) to give ethyl cyclopentanone-2-carboxylate. Indole-3-propionic acid was then formed by the procedure of Kalb, Schweizer, and Schimpf (1926) as modified by Manske and Robinson (1927).

The same authors prepared indole-3-pyruvic acid by applying the Grignard reaction to indole to give indole-aldehyde (Putochin's (1926) modification of the method of Majima and Kotake (1922): the method was further modified by Berg *et al.* in that di-*n*-butyl ether was used as a solvent). The aldehyde was converted to the pyruvic acid according to the procedure of Ellinger and Matsuoka (1920).

Recent Indole-Acetic Acid Syntheses

Two syntheses of indole-3-acetic acid and one synthesis each of indylene-1:3-diacetic acid and of two other indole acids, were reported by Jackson and Manske (1935). One of the indole-acetic acid syntheses was from diethyl- β -cyano-



propionacetal $\xrightarrow{\quad}$ acid $\xrightarrow{\quad}$ semi-aldehyde of succinic acid ($+\text{H}_2\text{O}$); this, without isolation, was condensed with phenylhydrazine; the product was subjected to the Fischer indole

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ring-closure, yielding the required acid. The other was a variation of that of Piccinini (1899), who used it to prepare N-methylindole-3-acetic and 2-methylindole-3-acetic acids: Jackson and Manske (1935) reported that a moderate amount of ethyl diazoacetate acting upon indole yielded mainly indole-3-acetic acid and no 2-substituted compound. "The isolation of the acid proved to be facile enough, and in some respects this synthesis is the most satisfactory yet recorded." Details of this synthesis are quoted from the original paper:

Procedure

"A dried ethereal solution of ethyl diazoacetate prepared from 50 gm. of glycine ester hydrochloride was slowly run into a Claisen flask (fitted with a long fractionating column) in which had been placed 19 gm. of indole and a trace of copper powder. The ether was rapidly distilled off as the solution was run in, so that the reaction was thus made to proceed smoothly. Finally the product was heated to 100°C. in the vacuum of a water pump, transferred to a Claisen flask, and distilled at 2 to 3 mm. There was frequently a second evolution of gas at this stage, and this was particularly pronounced in the experiment in which the proportionate quantity of diazoester was greatly increased. [This second experiment yielded less mono-acetic acid—H.N.]

The almost colourless distillate was hydrolyzed with an excess of methanolic potassium hydroxide, the solution diluted with water and the methanol distilled off. Thorough extraction of this solution with ether yielded 2 gm. of indole. The alkaline solution was then acidified with hydrochloric acid and again exhaustively extracted with ether. The combined extracts were dried with sodium sulphate and the ether distilled off. The solid, reddish residue was transferred to a suction funnel and washed with cold ether.

The indolyl-3-acetic acid readily dissolved in the ether and was recovered from the filtrate by removal of the solvent. It was recrystallized from a concentrated solution in ethyl acetate by the addition of benzene, or from hot water. The latter solvent is preferable when the acid is already of good

grade. In either case, the adequately purified product melted at 167° to 168°C. (corr.), with the evolution of carbon dioxide, and this melting point was not lowered when the material was admixed with a specimen prepared from indolyl acetonitrile or one synthetized by the method to be detailed later [*i.e.*, from the cyanopropionacetal mentioned above; not otherwise reproduced here—H.N.]. The yield of indolyl-acetic acid was 12 gm. and that of the diacetic 1·2 gm."

Indylene-1: 3-diacetic acid and the other two acids described in this paper (3-methyl-indolyl-1-acetic and indolyl-3-succinic acid) are much less effective physiologically than is indole-3-acetic acid. The very effective indole-3-propionic acid can, however, be obtained by heating the 3-succinic acid. Another synthesis of indole-3-acetic acid, and syntheses of other indole compounds, were recorded by King and L'Ecuyer (1934). Several of these compounds had carboxy in the 2-position. King and L'Ecuyer prepared the first 2-acetic compound (2-carboxy-1-methyl-indole-2-acetic acid). The simple indole-2-acetic acid is not known. Still another synthesis of indole-3-acetic acid is mentioned on p. 113.

Microbiological Preparation

Biological methods of preparation are suggested by the work of Ehrlich (1912). In presence of a yeast and much sugar, tyrosine gives tyrosol (*p*-hydroxyphenyl-ethyl alcohol), and Ehrlich discovered that tryptophan similarly gave the analogous alcohol (named by him tryptophol): in absence of other sources of nitrogen, the yeast deaminized the tryptophan. In the hands of Jackson, (1929) Ehrlich's "elegant biological method" gave a yield of over 90 per cent. of crude crystalline tryptophol, but Jackson did not say whether he used the sugar or alcohol technique. Jackson (1930) has prepared tryptophol artificially by reduction of methyl (or, ethyl) indole-acetate. As tryptophol is 3-indole-ethyl alcohol, it is curious that no laboratory oxidation of it to indole-acetic acid has been recorded, though its phenyl ether has been prepared by Manske (1931). A physiological oxidation of tryptophol to indole-acetic acid has been recorded by Ward (1923).

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Ehrlich's original method of preparation of indole-ethyl alcohol involved the use of tryptophan (which is expensive) and a large excess of sugar. Ethyl alcohol can be employed as source of energy for the yeast, the purification of the desired product being thereby made more easy than when sugar is used. Ehrlich (*loc. cit.*, p. 889) gave details of the preparation of indole-ethyl alcohol from tryptophan and ethyl alcohol in presence of a yeast. The requisite amount of alcohol is much smaller than the massive dose of sugar required for the sugar preparation.

It is not clear whether the ability to deaminize tryptophan in laboratory culture is peculiar to strains of yeast similar to those used by Ehrlich. The biological method, however, appears to have interesting possibilities and is probably susceptible of further refinement. It might be worth while to explore the potentialities of the microbiological deamination of casein or of its crude products of hydrolysis, the latter being obtained by either chemical or biological means. See also p. 103.

A substance probably identical with 3-indole-acetic acid has been formed in minute amounts by the action of legume nodule bacteria on tryptophan (Link *et al.* (1937); Chen (1938); Georgi and Beguin (1939)); it may help to account for the auximonic effect of leguminous nodules observed by Bottomley (1915-17, E).

Higher Indole-Acids

Details of a synthesis of 3-indolyl-propionic acid from $\omega\omega$ -diethoxy-propyl diethyl-malonate (from β -chlorpropionacetal and sodium diethyl malonic ester) are given by Davies, Atkins, and Hudson (1937). The acid prepared in this way is free from the lower homologue.

Manske and Leitch (1936) have described in considerable detail the syntheses of δ -(3-indolyl)-valeric acid and the related *bz*-alkyl- β -(5-methyl-indolyl)-propionic acid. Both these have been shown to possess the plant-physiological properties of a growth-regulating substance.

The indole-valeric acid was synthetized by the application

of the Fischer ring-closure to the phenyl-hydrazone of α -keto-suberic acid and the subsequent elimination of carbon dioxide from the resulting dibasic acid (δ -3-(2-carboxy)-indolyl-valeric acid). This synthesis was strictly analogous to that of the two lower homologues (Jackson and Manske (1930); Manske and Robinson (1927)), the phenyl-hydrazone being obtained by means of the Japp-Klingemann¹ reaction from the homologous ethyl cyclo-heptanone carboxylate. This was prepared following the method first used by Kötz and Michels (1906) for the synthesis of ethyl cyclo-hexanone carboxylate. For the 1936 paper, cyclo-heptanone (suberone) was obtained by the procedure (described as "excellent" by Manske and Leitch) due to Mosettig and Burger (1930).

In outlining the preparation of the *bz*-methyl indolyl derivative, Manske and Leitch wrote that "the now well-known procedure, starting with *p*-toluidine in this case and ethyl cyclo-pentanone carboxylate, was employed".

A number of other indole derivatives, mainly of purely chemical interest at present, have been prepared by Manske (1930), including the phenyl ether of tryptophol mentioned above. The "recently much exploited" reaction of Japp and Klingemann was used in all cases to prepare the requisite phenyl-hydrazones. Manske also referred to the synthesis by Barrett, Perkin, and Robinson (1929) of 5- and 6-methoxy- β (3-indolyl)-propionic acids by methods analogous to those of Manske and Robinson (1927). Manske himself (*loc. cit.*) synthetized the 7-methoxy-3-indolyl-propionic acid and a more complex *bz*-substituted indolyl-propionate.

An isomeric form of indole has been reported by Braun and Nelles (1937), but it is not known how it or its compounds affect plant growth.

Other Compounds

For preparation and properties of indole-carboxylic acids, see the textbooks. The 3-carboxy compound is easily converted into indole.

¹ The Japp-Klingemann (1888) reaction arises from the addition of benzene-diazonium chloride in ethyl alcohol made alkaline with KOH.

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Methyl-ketole (2-methyl-indole) and skatole (3-methyl-indole) converted into Grignard magnesium halogenides, form the respective 2- or 3-methyl-1-carboxy acids when treated at room temperature with CO₂. If the CO₂ is passed in at the temperature of boiling toluene, the magnesium halogenide of 2-methyl-indole forms only 2-methyl-3-carboxy-indole. The magnesium halogenide of skatole persists in yielding the 1(N)-carboxy compound when treated with CO₂ even at 200°C., and a bath temperature of about 310° is necessary for the carboxy group to become attached in the 2-position (3-methyl-2-carboxy-indole) instead of in the 1-position.

Indolyl magnesium halogenides commonly form 3-substituted alkyl derivatives from alkyl halogenides, though substitution in the 1-position may occur.

The preparation of many indole derivatives, including those mentioned in the foregoing paragraphs, and also 3-acetyl-indole (3-indolyl-methyl-ketone) and homologues, has been described by Oddo (1921). Some of his descriptions are reproduced in Houben's "Die Methoden der organischen Chemie," 2nd. edn., Leipzig, 1924; Vol. IV, p. 882.

1-methyl-indole-3-carboxylic acid, from treatment of methyl-phenylhydrazine-pyroracemic acid with hydrochloric acid: Hartley and Dobbie (1899).

1-methyl-indole-3-acetic acid: Piccinini (1899). See p. 106.

Few of the above-mentioned substances have been tested on plants, but they are mentioned for the sake of completeness in view of their possible synthetic or constitutional importance.

Still other synthetic compounds have been submitted to the *Avena* and other tests by Kögl and Kostermans (1935), Haagen-Smit and Went (1935 F) and others. Lists of their results are given in Boysen Jensen's book.

The growth-regulating value of salts of synthetic acids has

been very favourably reported by Avery *et al.* (1937), and by Zimmerman and Hitchcock (1937), whose paper on "Comparative effectiveness of acids, salts, and esters as growth substances, and methods of evaluating them" provides an interesting discussion.

Allo-cinnamic acid: see p. 84. *Glycerides*: see p. 81.

Naphthalene Compounds (See also p. 119).

Zimmerman and Wilcoxon (1935) prepared α - and β -naphthalene-acetic acids from the corresponding methyl-naphthalenes by bromination, conversion to the nitrile, and hydrolysis of the nitrile by the procedure of Mayer and Oppenheimer (1916). It may be noted that α -naphthalene-acetic and γ -indolyl-3-butyric acids are the most powerful root-forming acids known (Zimmerman, 1936). For the synthesis of indolyl-butyric acid, see Jackson and Manske (1930). Use of a pure α -naphthyl-acetic acid free from the β -form and from naphthoic acid present in earlier preparations, was reported by Zimmerman and Hitchcock (1937). This was presumably the same as the acid prepared by Wilcoxon (1937) by a procedure based on Rousset's (1897) method. Employing the Friedel and Crafts reaction between naphthalene and ethyl chloroglyoxylate, Rousset obtained α -and β -ketonic esters in yield 50 per cent. of theory; reduction of the ketone acid then yielded the corresponding glycollic or acetic acid.

Wilcoxon's paper reviews probably all the existing methods for the preparation of naphthyl-acetic acids. It is of interest to note that Wilcoxon was unable to obtain any naphthyl acid by following the patented procedure of Wolfram *et al.* (1934).

His modification of Rousset's method gave a product (yield 47 per cent. of theory) consisting entirely of the desired α -form of ethyl naphthalene-glyoxylate.

Reduction of this with sodium amalgam gave a 40 per cent., and with hydrogen and nickel catalyst a 88 per cent., yield of α -naphthalene-glycollic acid. Reduction of the glyoxylate ester with hydriodic acid and red phosphorus gave a 90-per cent. yield of α -naphthalene-acetic acid.

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The ethyl naphthalene-glyoxylate, and the naphthalene-glycollic and -acetic acids were stated to exhibit plant growth-regulating properties in varying degrees, but the details of the physiological experiments have not yet been published.

Higher Aryl Compounds

These are included in the only set of examples of the production of growth-regulating substances protected by patent (Wolfram *et al.*, 1934).

Anthracene-acetic, fluorene-acetic, and acenaphthene-5-acetic acids can be prepared by heating the hydrocarbons with chlor-acetic acid. The yields are small, but Zimmerman and Wilcoxon (1935) found that they could be improved by adding aluminium chloride to the melt. The location of the substituents in the anthracene and fluorene compounds is not known.

An Important Hint

In this series of indolyl and related syntheses it has been customary to aim at the preparation of the pure acids, often via an alkyl ester subsequently hydrolyzed. In view of work by Zimmerman, Hitchcock, and Wilcoxon (1936) it is doubtful whether the sometimes troublesome isolation of the free acid is necessary or desirable. These authors have shown that several esters belonging to the series of phenyl-acetic, naphthyl-acetic and indole-fatty acids are quite effective in initiating or otherwise affecting growth of organs and cells in plants. Methyl 3-indole-acetate indeed, was more effective in this way than was the free acid: the "natural" "hetero-auxin". The same authors state that there is no evidence at hand that hydrolysis of these esters to acids occur in plant tissues. It has, however, been shown by Zimmerman and Wilcoxon (1935) that α -naphthalene-aceto-nitrile was not only not poisonous to plants, but was very effective physiologically in the same respects as was the free acid derivable from it by hydrolysis in the laboratory.¹

¹ The nitrile was more effective in root-production on stems of large plants than was the free acid at the same concentration.

Synthesis

No record of a plant physiological test of indole-3-acetonitrile seems to have been made, but Jackson (1929) secured 13 gm. of the nitrile from 15·6 gm. of indole in the course of preparing indole ethylamine by the method of Majima and Hoshino (1925).

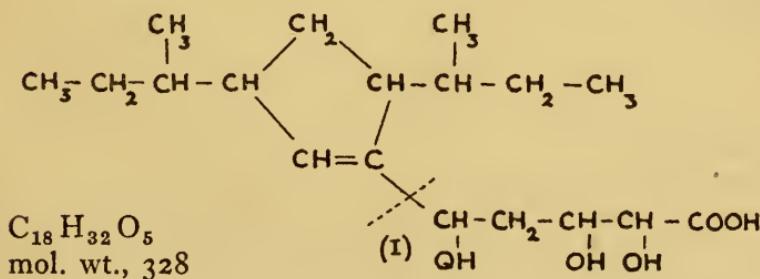
The synthesis of Majima and Hoshino represents a quite practical method of preparing indole-3-acetic acid: indole magnesium iodide, treated with chlor-aceto-nitrile in ethyl ether or in anisole solutions, yields the nitrile. The nitrile is easily hydrolyzed to the acid.

As some of the acids, more particularly the naphthyl-acetic acids, are prepared via the corresponding nitrile, we have an additional reason for believing that isolation of an acid is not an essential goal in the manufacture of a potent growth-regulating substance.

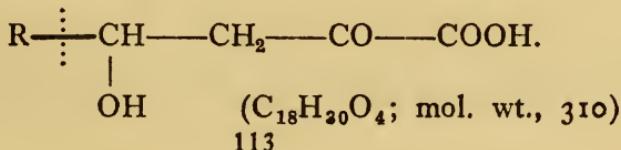
THE AUXINS

These are true plant hormones. They have not yet been synthetized. Their structure was established by the work of Kögl (1935).

The formula of auxin-a (*syn.*, auxentriolic acid) is:



The configuration of auxin-b (auxenolonic acid) is similar, the side chain (I) being replaced by: ($\text{R}=\text{C}_{18}\text{H}_{23}$)



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Auxin-a is very stable towards acids, but is readily decomposed by alkalis. Auxin-b is decomposed by both acids and alkalis, whereas indole-acetic acid, though hydrolyzed by acids, is very stable towards alkalis. These properties were made use of by Kögl, Haagen-Smit, and Erxleben (1934) as showing that the growth-hormone in the tips of oat seedlings was probably auxin-a.

Auxin-a lactone, distilled with potassium hydrogen sulphate, yielded a substance probably identical with auxin-b. (Kögl, 1935).

Kögl (1938) has pointed out: "The formula of auxin-a contains seven asymmetric carbon atoms; any organic chemist knows what that implies. Suppose it were possible on starting from inactive material to produce an auxin-a of the correct structure, even then the product would consist of a mixture of 64 racemates, requiring to be resolved into 128 optical enantiomorphs. And only one component of that mixture of stereoisomers would be identical with auxin-a."

Synthesis of auxin-glutaric acid (containing four asymmetric carbon atoms) has been accomplished, and a product identical with the natural derivative was obtained after about 300 fractionations, so that definite proof of the constitution of a 13-carbon-atom part of auxin-a and -b has been obtained (Kögl (1938)).

VITAMINS

Vitamin B₁

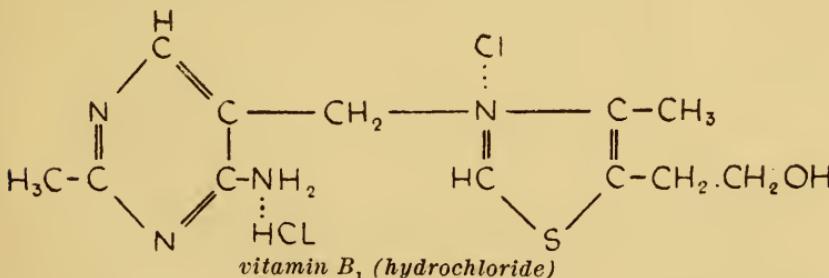
Although when this book was first planned, the idea of a synthetic plant growth-substance did not extend beyond the phenyl, indolyl, naphthyl, and related compounds introduced into horticultural use about 1935, at least two of the vitamins, and perhaps other synthetic substances, have or are likely to have considerable horticultural importance. (P. 80).

The complete synthesis of vitamin B₁ was accomplished, or at least published, almost simultaneously in 1937 by American, British, and German workers. The question of priority has been discussed in letters from Robert Robison and A. L.

Bacharach in *Chemistry and Industry*, 1937, 56, 1141. Vitamin B₁ is called *aneurin* by many British and French workers, and *thiamin* by many Americans. Its structure is much more complicated than that of the indolyl plant growth-substances, to which it has no chemical resemblance.

In plants, it is present, particularly in the embryo of seeds, and it appears to act as a hormone within plants. According to much work by P. R. White and others (summarized in *Chronica Botanica*, 1939, 5, 166) vitamin B₁ is an essential ingredient of media for the culture of excised roots and other parts of dicotyledonous plants¹. Its hormonal rôle in the intact plant does not seem to have been ascertained. Applied externally to cuttings, and possibly to seeds, vitamin B₁ behaves as a drug, and, like the synthetic plant growth-substances of the indolyl group, induces or hastens root-formation. It is possible that some of the effects observed from applications of preparations of maize and other seeds to seeds and cuttings—particularly by Russian workers, were due to vitamin B₁, and not to auxin as was thought. Vitamin B₁ has been found in dung (see p. 68), wherein it is probably formed by micro-organisms.

The formula of vitamin B₁ is :



Vitamin B₁ is now synthetized in America, Britain, and Germany by the processes discovered in these respective countries, and is on sale. The German process has been patented.

¹It is not always necessary to add the complete substance, as some organisms can synthetize the substance by condensation of its single-ring constituents, or from an appropriately substituted thiazole alone (Robbins *et al.*, (1937)).

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REFERENCES

Synthesis of Vitamin B₁

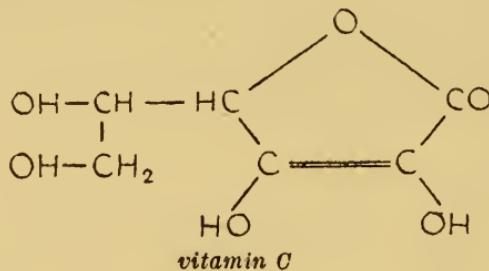
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Vitamin C

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Its formula is:



Vitamin B₆

This, like nicotinic acid and other substances, is sometimes useful in culture of excised roots (see White, above). It is mentioned for the sake of completeness.

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CHAPTER XIV

IDENTIFICATION OF GROWTH-SUBSTANCES AND SUBSTANCES RELATED TO THEM

Animal substances are much more compound than those we have hitherto examined. Several of the substances furnished by animals still preserve many of the properties of the vegetables by which they are nourished.—BEAUMÉ.

THE following selected information is given in the hope that it will be useful to those proposing to undertake the search for growth-substances in agricultural materials such as manure and compost. As no work has yet been done along such lines (except for the early isolations from decomposed meat) the available information will require to be specially adapted to suit each worker's need. The existing qualitative tests refer entirely to the identification of the pure substances in aqueous solution or as they occur in urine. When urine is mentioned here, it is usually human urine that is implied.

Critical methods of identifying the growth-substances are urgently required. No progress can be made in the recognition and estimation of the substances in organic manures and decomposition products until simple yet accurate chemical tests are available. Much of the information that is available is published in medical and physiological journals difficultly accessible to agricultural and other chemists. The handbooks on organic chemistry give very little help of the kind useful for performing analysis of extracts, and it seems that the detection of ring-compounds in manure and composts will remain for some time a matter for the research laboratory able to isolate the substances for characterization by such criteria as melting points. (See Tabular Index, Chapter XV). Such methods are not only laborious, but are seldom suscep-

table of quantitative application. Physiological tests can supply roughly quantitative information in some cases at high dilutions, but are of little critical value in distinguishing the constituents of mixtures.

Phenyl-acetic acid.—Steenbock (1912) has described a method for the estimation of phenyl-acetic acid in urine in presence of benzoic acid. He treats the urine with caustic soda with addition of hydrogen peroxide, extracts the benzoic and phenyl-acetic acids with ethyl ether, dries, and weighs after sublimation. The sublimate, of weight S grams, is titrated with $N/20$ NaOH, requiring n ml. The phenyl-acetic acid content in grams is then:

$$\frac{136 (S - 0.0061 n)}{14}$$

The acid is oxidized to benzoic acid by strong oxidizing agents. It has a beautiful rose-like odour. For other tests, see the chemical handbooks.

Phenyl-propionic acid. (β -phenyl-propionic acid).—For tests see chemical handbooks. It has a pleasant, sweet odour.

Phenyl-pyruvic acid (not shown to be a growth substance) has been found in the urine of a small proportion of mental defectives by Fölling (1934). Urine containing this substance gives a deep green colour on addition of ferric chloride solution. For other properties and quantitative methods, see the paper by Penrose and Quastel (1937). Like homogentisic acid (see *infra*) phenyl-pyruvic acid is a product of incomplete metabolism; its source appears to be dietary phenyl-alanine. It is fairly easily decomposed under alkaline conditions in the presence of bacteria in urine, but the products of such decomposition are not known.

p-hydroxy-phenyl-acetic acid.—With ferric chloride solution, its solution gives a faint violet colour, quickly becoming greyish-green.

p-hydroxy-phenyl-propionic acid (β -(4-hydroxy-phenyl)-propionic acid).—Said to occur in urine. The cupric salt is difficultly soluble in water and alcohol, but is soluble in ether. (Hlasiwetz, *Journ. prakt. Chem.* (1), 72, 401; from Beilstein's

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Handbuch). The acid gives a deep red colour with Millon's reagent.

Tyrosine (*p*-hydroxy-phenyl-alanine).—Not a plant-growth substance while intact. For tests, see textbooks.

For properties and methods of isolation of some aromatic hydroxy-acids, see the sections by Waser (1923), and by P. Brigl, in Abderhalden's *Handbuch*, 1931, Abt. 4, Teil 5(1), 483-512, and Baumann's (1882) method on pp. 510-512 of the latter.

Homogentisic acid. (2:5-dihydroxy-phenyl-acetic acid.)—The claim of this body to be considered a growth-substance is not certain, but it may be useful to give a few hints for its detection and estimation, as a guide to adoption of methods to search for it in agricultural materials. Readers who are especially interested cannot do better than consult Weise's monograph (see p. 75).

From urine containing homogentisic acid and shaken with sodium hydroxide solution and benzoyl chloride, di-benzoyl-dihydroxyphenyl-acetamide ($C_6H_5\cdot COO)_2\cdot C_6H_3\cdot CH_2\cdot CONH_2$ separates out, which can be recrystallized from hot ethyl alcohol. M.P. 203-204°. This derivative is characteristic of alcaptonuric urine, but needs to be differentiated from tribenzamide, M.P. 201-205°, which is liable to form in small amounts when urine is benzoylated. Tribenzamide, however, is difficultly soluble in hot alcohol.

The lead salt of homogentisic acid is useful for isolation; it crystallizes in colourless or brownish prisms of M.P. 214°. It is insoluble in alcohol and ether, and only very slightly soluble in water.

With ferric chloride solution, homogentisic acid, even in fairly dilute solution, gives a transient blue colour. With Millon's reagent, an aqueous solution of the acid gives a yellow colour; on standing, a yellow precipitate develops in the cold, which turns red on heating.

A peculiar titration method has been evolved by Baumann (1891). This depends on the reduction of N/10 silver nitrate solution in urine made alkaline with ammonia. To 10 ml. of urine are added 10 ml. of 3 per cent. ammonia solution, and

at once "a few" ml. of standard silver nitrate solution. The mixture is shaken, and allowed to stand for five minutes. Five drops of a 10 per cent. solution of calcium chloride and ten drops of a 20 per cent. solution of ammonium carbonate are then added, in order to form a precipitate of calcium carbonate to carry down suspended silver. The mixture is filtered, and the filtrate tested with silver nitrate. If appreciable reduction occurs, a new experiment is begun, using 2 to 5 times as much standard silver nitrate solution as first. The reduction test is repeated, and the whole thing is gone through several times, if necessary, until no reduction occurs in the filtrate. This gives an upper limit to the volume of standard silver nitrate. The experiments must again be repeated *ab initio* with successively slightly smaller doses of standard silver nitrate until no reduction occurs on the addition of silver nitrate to one portion of the filtrate, and also no or very slight cloudiness is revealed on the addition of excess hydrochloric acid to another portion. Each ml. of standard silver nitrate solution that has produced this limiting result is equivalent to 4.124 mg. of homogenetic acid. The method is probably capable of simplification.

Indole-acetic Acid. The Urerosein Test

A number of chemical reactions are available for revealing the presence of indole-acetic acid. They have been described in the literature as tests for the substance in urine, or in fairly concentrated pure solution. Some tests for the pure substance follow.

E. Salkowski's tests date from 1884 and are the best known. (1) If a 0.1 per cent. neutral solution of the acid is treated with a little very dilute ferric chloride solution, the mixture becomes cloudy; in transmitted light it appears violet, but greyish in reflected light. On careful acidification a violet-grey precipitate is formed, which gives a blue solution in ethyl alcohol.

If after adding ferric chloride and acid, more ferric chloride is added and the mixture is boiled, a cherry-red colour is produced. The colour can be removed by extraction with

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amyl alcohol, in which the red substance is very soluble.

(2) If to the neutral solution of the acid is added an equal volume of hydrochloric acid (D. 1.12) and a few drops of about 1 per cent. "bleaching-powder solution", a purple-red colour is produced. In concentrated solutions a precipitate is formed, soluble in ethyl alcohol and in amyl alcohol.

Ehrlich's test.—Resembling the usual diazotization reaction in hydrochloric acid solution, this is performed with alcoholic *p*-dimethylamino-benzaldehyde in hydrochloric acid in presence of nitrite. With indole-acetic acid a reddish colour is produced, not very distinct from that given by indole.

Lead acetate gives a crystalline precipitate with a 0.1 per cent. solution of sodium or potassium indole-acetate, whereas other heavy metals give little or no precipitate (Ellinger).

Indole-acetic acid is not volatile in steam, is stable towards alkalis, and is odourless.

Link *et al.* (1937, H) used the Salkowski colour test as sole chemical test for indole-acetic acid. They remark that it is considered specific for that compound. Evidence for its specificity is not quite satisfactory. An analytical scheme for the detection of the principal indole-acids and related compounds is badly needed.

Urorosein is a derivative of indole-acetic acid. It was discovered by Nencki and Sieber in 1882. Opinions differ about its constitution.

Weise in Abderhalden's *Handbuch*, Abt. 4, Teil 5(1), gives tests for the presence of indole-acetic acid (p. 767) and for the production of urorosein (p. 768) which, though similar, differ in detail. It has been suggested that urorosein is a derivative of indolyl-aceturic acid (indolyl-acetyl-glycine), the conjugation product (see p. 58) of indole-acetic acid, but it appears uncertain that the glycine takes part in the production of urorosein.

The tests given by Weise (*loc. cit.*) are as follows:

"*Indole-acetic acid* (Salkowski's test).—If a few drops of pure nitric acid (D. 1.2) are added to a very dilute solution

of indole-acetic acid, and then a few drops of 2 per cent. potassium nitrite solution are added, a cherry-red colour appears; if the dilution is not too high, a red colouring-matter is precipitated, which is easily soluble in ethyl acetate and in amyl alcohol. Caustic soda solution discharges the colour from ethyl acetate solution and acquires a yellow tinge. The alkaline solution is rendered colourless after addition of zinc dust and remains colourless on exposure to air. (Distinction from indole; nitroso-indole after reduction becomes blue on standing—Salkowski.)”

The same test is given by Ellinger on p. 792 of Teil 7, Abt. I, of Abderhalden's *Handbuch*. (Urorosein may often be formed from urine by simply adding hydrochloric acid; its formation requires the presence of nitrite, which is often present in urine owing to bacterial activities). The following will now be understandable.

“For the demonstration of urorosein, 5–10 ml. of concentrated hydrochloric acid are added to 50–100 ml. of urine. If no red colour appears after a few minutes, a little 0·2 per cent. sodium nitrite is cautiously added, drop by drop. The red colour then appears if the chromogen is present.

“The dyestuff thus formed can be separated out by shaking with amyl alcohol: this operation is especially necessary when other red or yellowish-red colouring-matters are present. The amyl alcohol solution should be examined spectroscopically.

“Urorosein dissolves to a reddish solution in water, ethyl alcohol, amyl alcohol, but is insoluble in ether and in chloroform (distinction from indirubin and skatole red). The colour of the solutions is discharged by addition of alkalis, and restored on acidification. The amyl alcohol solution shows a sharp absorption band in the green, about 557 Å.U.”

From Weise's account of the indole-acetic acid tests with acid and nitrite, it looks as if nitroso-indolyl-acetic acid

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is the product. Apparently, urorosein and the indole-acetic acid nitritatior compound behave differently with alkalis. On account of the apparent absence of information regarding the behaviour of urorosein in ethyl acetate, the question of the identity of the two substances in question must be left open. Plimmer (1918) wrote that urorosein is most probably nitroso-indole-acetic acid. Ellinger and Flamand (1909) and Riesser (1911) believed urorosein to belong to the group of tri-indolylmethane dyes discovered by Ellinger and Flamand; Weise (*loc. cit.*, p. 768) imputed to H. Fischer (1923) the view that it is a di-indolyl-methene derivative. Urorosein has also been studied by Herter (1908).

Schmitz (1931), apparently quoting Riesser (1911), wrote:

“Eine Darstellung des Uroroseins in Substanz ist bisher nur auf synthetischem Wege durch Oxydation von Indole-essigsäure mit Eisenchlorid oder mit Natriumnitrit und Salzsäure versucht worden.”

Further pursuit of the subject is left to those particularly interested.

With the modified Adamkiewicz-Hopkins (tryptophan) reaction of Winkler (1934), indole-acetic acid gives a reddish violet colour. The best ratio of reagents is 1 mol. of indole-acetic acid, 1 mol. of glyoxylic acid, 2 mol. Cu (sulphate or acetate) and excess of concentrated sulphuric acid—added in that order. (Winkler and Petersen, 1935).

For titration-curves of 3-indole-acetic, -propionic and -butyric acids in distilled water, see the paper by Albaum and Kaiser (1937).

A study of three colorimetric methods of estimating indole-acetic acid in aqueous solution has been made by Mitchell and Brunstetter (1939).

See also Lefèvre (1939, G).

Methods of Isolation of indole-acetic acid

The *Avena* (oat) test has been used by Kögl *et al.* (1934) as a method of assay of indole-acetic acid in fractions of urine during the process of isolation. The normal urinary excretion

of growth-substance was estimated by them at about 2 mg. per day, of which only about a fifth is indolyl-acetic acid, the remainder consisting chiefly of auxin-a. In the case of their special patient, "E.W.V.", who excreted 8-10 mg. of growth substance per day, at least three-quarters of that amount consisted of indole-acetic acid.

The oat assay of the successive urine fractions showed very satisfactory agreement with the determinations of indole-acetic acid by weight, allowance being made for losses and for the presence of auxin in the original urine. (The oat-test activities of the two auxins and of indole-acetic acid are very similar).

Weise (*loc. cit.*) gives the following method of preparation of indole-acetic acid from urine. It may perhaps serve as a basis of a method of isolation from agricultural materials.

Several litres of urine are evaporated down, and the residue extracted with absolute alcohol. The extract is distilled, and the residue is dissolved in a little water and acidified with sulphuric acid. It is then shaken with ether. The ether extract is separated, and is shaken with a sodium hydroxide solution to remove the indole-acetic acid. The alkaline solution is evaporated down, and the residue is repeatedly extracted with absolute alcohol to a total volume of about 100 ml. Ether is then added, and a precipitate forms. The precipitate is separated from the liquid, the latter is evaporated down, and the residue is acidified with hydrochloric acid. After an ether extraction of the acid mixture, the ether is evaporated off. This gives an impure product (containing traces of hydrochloric acid) which can be dissolved in hot benzene and recrystallized.

Kögl and others (1934, and other papers) have improved on Weise's method in several respects, notably by taking up the indole-acetic acid on an absorbent, thus avoiding the tedious evaporation. They have also made use of a chromatographic technique to separate the indole-acetic acid from auxin. Their papers should be consulted for details.

E. Salkowski's original methods of isolation of indole-acetic (1884) and indole-propionic acid (1899) from decom-

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posed meat, on account of the presence of indole and other substances in the mixture, are too complicated to be given here, but they should be borne in mind.

Indole-propionic acid.—Ferric chloride added to a dilute solution of the acid gives a white turbidity, which becomes red on heating. Concentrated potassium nitrite and acetic acid added to a saturated solution in water form fine yellow needles of a nitroso compound, which melts at 135° with evolution of gas.

Tryptophan.—Not a growth-substance, when intact. For usual tests see textbooks.

An important modification of the well-known Adamkiewicz-Hopkins reaction has been made by Winkler (1934). This reaction depends on the blue-violet colour developed when glyoxylic acid (often present as an impurity in acetic acid) and concentrated sulphuric acid are added to free or combined tryptophan solutions. Winkler has shown that the test is inadequately given, or even fails, unless a trace of copper salt (conveniently, sulphate or acetate) is added before the sulphuric acid. The glyoxylic acid should be roughly equimolecular with the tryptophan. Under conditions described, the modified reaction is quantitative for free tryptophan at least.

For a method of detection of free and combined tryptophan in plants by nitration, see Roth (1939).

Creatine and creatinine.—For properties and tests, see textbooks on human biochemistry. Creatinine was recognized by Schreiner *et al.* (1911, E) by means of its zinc chloride compound.

Oxal-acetic acid.—This is not known to be a growth-substance in the current sense, but it is assumed by Virtanen and Laine (1937) to be an intermediate in the biological fixation of nitrogen, particularly in leguminous plants. In either aqueous or alcoholic solution, both forms of the acid give an intense red colour with ferric chloride solution, wherefore it may perhaps be confused with indole-acetic acid. Oxal-acetic acid, however, has a peculiar reaction : in presence

of aniline, both of its forms produce a lively evolution of carbon dioxide (Wohl, 1907), even at temperatures as low as 10° . Both forms are insoluble in chloroform and benzene (distinction from indole-acetic acid), but the higher-melting form is said to be more soluble in ether than the other is.

Vitamins

Chemical methods for the estimation of vitamin C are reviewed by Gstirner (1939), who considers the foremost method to be the standard one of J. Tillmans (*Chem. Abs.* **25**, 5692; variously modified since). What appears to be a more reliable method for determining ascorbic acid in urine has been described by Roe and Hall (1939).

For a biological method of determination of vitamin B₁ in urine by its effect on growth of the fungus *Phycomyces*, see Villela (1938); for the original *Phycomyces* method, see Schopfer and Jung (1938). For a fluorescence method of estimation of vitamin B₁, see Marrack and Höllering (1939).

Went's Pea Test

A simple test for the presence of at least some growth-substances has been described by Went (1934). It was devised to test for the presence and rough quantitative estimation of auxin, but works equally well with indolyl-acetic acid.

Peas are germinated in any convenient manner (on damp cloth, sand, or blotting paper) in a darkened cupboard or room maintained at about 25° C. No nutrient solution is required, and small amounts of light do not matter so long as the shoots grow and remain straight. When the shoots are from 5 to 20 cm. long they are ready for use. The variety of peas mentioned by Went was *Alaska*.

The description that follows is given in Went's words:

"Pieces of stem 2 to 20 cm. long are cut about 5 mm. below the terminal bud, and are used either directly or 4 to 8 hours after cutting. Immediately before application of the auxin solutions, the top of the cut piece of stem is split lengthwise by an exactly median cut with a razor

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blade over a length of 1 to 3 cm. If the cut is very far from median the curvatures are smaller. Upon subsequent immersion in the solution the two halves will bend out because of the tissue tension. If no auxin is present in the solution these original outward curvatures do not change any more and are fixed, since they do not go back upon plasmolysis. In the presence of auxin, however, about an hour after immersion (at 25°) the free ends of the two halves will start to bend inwards; the higher the concentration the further they bend. After about 6 hours the final stage is reached, and the originally most rapidly growing zone will be convex at the outside. If the median slit is long enough, the halves will each show an S-shape. Also, these curvatures are not changed by plasmolysis. For the appearance of the curvatures it is only necessary to have the apical cut surface in contact with the auxin-containing solutions. So either the shoots are reversed and placed upside down in a small amount of solution (5 plants together in 0.2-1 cc.) or they are left floating on the surface of the solution in a shallow dish. If the auxin can enter only through the surface of the radial cut no curvatures appear.

"The simplest quantitative auxin determination with the pea test is to place a number of pea shoots, apically slit lengthwise over a distance of \pm 10 mm. in a whole range of dilutions of the solution to be tested; 5 plants per dilution will be enough. After 12 hours it is determined in which concentration a well-recognizable reaction occurs. In this way either directly or by interpolation the critical concentration may be calculated with an accuracy of 50 per cent."

This critical concentration, or difference of concentrations, corresponds to about 480 AE (*Avena* units), or 1.4×10^{-5} mg. of auxin-a, per ml. For purposes of comparison, it may be mentioned that ordinary human urine contains, per litre, about 2 mg. of auxins and indolyl-acetic acid together, each having an activity of about 50,000,000 AE per mg. Hence, Went's test is sensitive to the amount of growth substance present in about one-hundredth of a millilitre of urine.

Went says: "The only work involved in this method is the making up of the dilutions and the splitting of the peas; the estimation of the reaction is done at a glance."

The curvatures can also be classified. Went's scheme is:

<i>Degree of reaction</i>	<i>Appearance of Split Stems.</i>
0	No auxin reaction, both halves concave at the outside over their whole length. (as A, Fig. 6.)
1	Slight auxin reaction, trace of convexity in limited regions.
2	Definite auxin reaction, ends of both halves approximately parallel.
3	Fair auxin reaction, ends bent inwards. (as B, Fig. 6.)
4	Strong auxin reaction, ends just touching.
5	Very strong auxin reaction, both halves crossing at end.
6	Very strong auxin reaction, halves crossing midway or at base (as C, Fig. 6).

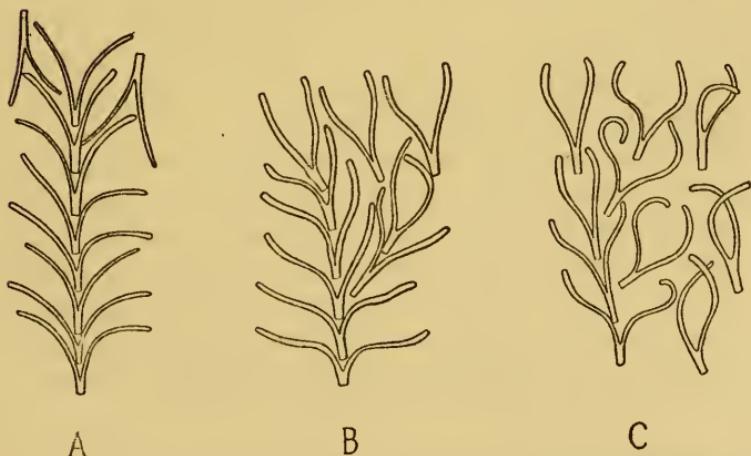


Fig. 6. (After F. W. Went, (1934)). Curvatures in split sections of pea stems, immersed in:

A, water.
B and *C*, auxin-containing solutions producing curvatures of degrees 3 (fair) and 6 (very strong) respectively.

With acknowledgments to Dr. F. W. Went and to the Editors of the
Verslag. Kon. Akad. Wet., Amsterdam.

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The crux of the preparation lies in the apparently simple recommendation to germinate the peas on a damp material. Germination is easy to accomplish, but peas are very prone to invasion by micro-organisms, often borne on the seeds themselves, and unless suitable treatment is applied, the experimenter is liable to the disappointment of finding the seedlings as a pulpy, stinking, mass. It is hoped that the following suggestions will anticipate for once the reproach that textbooks give all the details except the essential ones.

The pea seeds (*Alaska*) should be externally sterilized. This may be accomplished by shaking the peas in any easily-removable disinfectant, such as free chlorine, or by washing in 95 per cent. alcohol followed by 0·2 per cent. aqueous mercuric chloride solution (not in reverse order!). A simple and effective method is to cover the peas with concentrated sulphuric acid (pure oil of vitriol), and leave them in it for five minutes with occasional shaking. A conical flask of large capacity is best. Whatever method of sterilization is adopted, rinsing should be performed with several changes of water. Tap water will do, as absolute sterility is not necessary. If the sulphuric acid method is used, the first addition of water to the peas should be made rapidly and in great excess, with avoidance of local overheating (for the sake of the peas), and of spurting (for the sake of the operator).

Sand is perhaps more conveniently freed from unwanted organisms than are cloth and paper, which are best sterilized by autoclaving or baking. Heat-sterilization of large amounts of sand is a tedious process of uncertain effect. A sufficient degree of freedom from micro-organisms can be attained by the use of strong chemicals, such as are available to many workers not possessing an autoclave. Here again, any reasonable method can be adopted. The following technique gives satisfactory results:

Sand *q.s.* is placed in a stout-walled shallow vessel. Better than the usual laboratory ware, a glazed "china" pie-dish fills all requirements. The sand is treated with a hot solution of potassium permanganate acidified with sulphuric acid (5 or more grams per litre of each substance, and 60° C.) and

well stirred with a rod or strong spatula; the solution is renewed if necessary. After washing out the solution until only a faint pink colour remains, a slight excess of powdered sodium bisulphite is added, and the sand is intimately mixed under warm water—most effectively by using clean hands. It is important that no brown stain should be left on the sand; securing its removal demands some care. The sand is finally washed under a slow stream of running tap water, care being taken to manipulate so that every part of the sand is washed. The washing must be intimate, and need not be prolonged: mere running water will not penetrate far. Most of the excess of water being quickly drained off, the sand is ready for use. The peas should be sparsely placed, uncovered, on the more than saturated sand, and should be sprinkled with water, and inspected twice daily for the first few days of germination.

Possibly owing to the production of a volatile growth-substance, a decided curliness tends to become manifest in crowded pea shoots, hence a ventilated cupboard or dark room is better than a closed incubator, but the temperature should be maintained at about 25° C., and if small amounts of light gain entry, the vessels should be turned frequently. Some few of the peas are almost sure to turn mouldy; as soon as their condition is noticed, these should be removed with flamed forceps, and dropped into a bath of disinfectant of low surface tension. The germination cupboard should be disinfected after each crop has been grown. Time for a crop, about ten days.

Those who are thinking of performing this pea test as a research method will not need advice about its application, but those who desire merely to make a demonstration may be grateful for the hint that salt action and other effects may interfere with the test. For example, the author has found that after floating the split fragments of pea-stem on 20 ml. of liquid in a Petri dish, there was no certain response with concentrated urine, or with stale urine at any dilution. Fresh urine gave the maximum response of which it was capable—not, of course, the maximum of Went's scale—when about 4 ml. were diluted to 20 ml. Here, however, is a dislocat-

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ing snag for the unwarned non-biologist. Tap water (if not chlorinated) or water from a silver-lined or glass still should be used as diluent. Water from a copper still contains enough copper to ruin many experiments with even intact plants, and these tender pea preparations are very easily hurt. My parting word to my fellow chemist is: Remember in doing any work with plants, that you are handling a structure of wonderful complexity, and alive.

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A review of chemical methods of determining carotene and vitamins A, B₁, B₂ (lactoflavin), and C, including some methods adapted for urine analysis, has been given by K. Hinsberg in *Zeitschr. anal. Chem.*, 1939, **117**, 284. See also p. 68 for work with vitamin B₁.

ENVOI

As to author's afterthoughts and the more or less sincere apologies that often find place in a preface, I have little to say. *Qui s'excuse, s'accuse.* Only one point should be mentioned that cannot be fairly introduced elsewhere. It is that but little reliance has been placed on abstracts and references at second-hand except as regards some analytical data; most of the sources quoted have been seen in the original by me, though possibly a smaller proportion for the second than for the first edition.

Another function of a preface is to be the expression of the author's acknowledgments, a sort of grace before meat: often scamped by the reader anxious to get to the meal—though, to be sure, it is the author's grace and not the reader's. I think that the following acknowledgments will be better noted because they come at the end:

To Dr. George T. Moore, Director of the Missouri Botanical Garden, St. Louis, Missouri, U.S.A., for kind permission to reproduce Fig. 1 from the "Bulletin" of the Garden.

To Dr. A. E. Hitchcock, of the Boyce Thompson Institute for Plant Research, Yonkers, New York, U.S.A., for kind permission to reproduce Figs. 2, 3, 4 and 5 from the "Contributions" of the Institute.

To some senior colleagues of Rothamsted Experimental Station, for having read and criticized portions of the manuscript of the first edition (the blame for any mistakes remaining mine, of course), and finally:

To Dr. H. L. Pearse and the Imperial Bureau of Horticulture, both of East Malling, Kent, for courtesies in connexion with the supply of references.

CHAPTER XV

TABULAR INDEX

*of Principal Substances Mentioned (other than intermediates)
with Outline of Tests and some Physical Properties*

The late M. Geoffroy the physician was the first who thought of comprising in a table the fundamental relations or affinities in Chemistry.—BEAUMÉ.

THIS table has been arranged in alphabetical order under each main heading of Miscellaneous, Phenyl, Indolyl, Naphthyl, and Other Aryl Compounds.

Solubilities are indicated qualitatively as follows: in water (w...), ethyl alcohol (a), ethyl ether (e), acetone (t), ethyl acetate (s), benzene (b), chloroform (c), methyl alcohol (m), usual fat solvents (f)—:

Almost insoluble in cold solvent: w

Appreciably soluble in cold solvent: *w*

Appreciably soluble in hot solvent: *W*

Very soluble in hot solvent: *W*

Almost all the substances are solid or oily at ordinary temperatures, and only one boiling-point has been given.

* An asterisk indicates corrected melting-points.

(With acknowledgments to Beilstein's *Handbuch*, Heilbron's *Dictionary*, and other authorities.)

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Miscellaneous Compounds, including Inorganic and Aliphatic Substances, those of Unknown Constitution, and some trivial names

Those marked * are hypothetic substances.

Acetic acid	Effects on plants, etc. page	Chemical and other tests. page
"Adco" manure	9	—
Amino-acids (general)	26, 62, 104	—
Aneurin (<i>see</i> Vitamin B ₁)		
Ascorbic acid (<i>see</i> Vitamin C)		127, 129 seq.
Auxins (general)	5-9, 53, 71, 85,	
	91, 96, 113	
Auxin-a	5-7, 77, 78, 88,	114, 129 seq.
	113, 127	
Auxin-b	5, 113	114
Caulocaline*	—	—
Charcoal	36	—
Co-enzyme R	92	—
Creatine	63, 88	—
Creatinine	63, 88	128
Dihydro-folliculin	56	—
Dihydroxystearic acid	65	—
Ethylene	28, 77	—
Florigen*	54, 96	—
Formic acid	57	—
Heteroauxin (<i>syn.</i> indole-acetic acid, <i>q.v.</i>)	10, 27, 37, 91, 98, 99, 112	—
Honey	45	—
Magnesium compound	45	—
Manganese compounds	43-45	—
Mercurial seed disinfectants	46	—
Nicotinic acid	88, 116	—
Ornithine and compounds	71, 75	—
Oxalacetic acids	128	128
Pantothenic acid (composition unknown)	91	—
Phenol(s)	72	—
Phyllokaline*	—	—
Picoline compounds	75	—
Potassium permanganate	43-45	—
Pyridine compounds (<i>see</i> picoline)		
Rhizocaline*	26, 44, 60	26
Rhizopin (<i>syn.</i> indole-acetic acid, <i>q.v.</i>)	62	—
Sugars	43, 44, 81	—
Talc	46	—
Thiamin (<i>see</i> Vitamin B ₁)	—	
Urorosein (<i>see also</i> indole-acetic acid)		98, 123
Uvitonic acid	65	—
Vitamins B ₁	55, 61, 68, 80,	68, 129, 135
	81, 115, 116,	
— B ₆	116	—
— C	33, 68, 80, 93, 116	129, 135

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Phenyl Compounds

$(R = C_6H_5)$

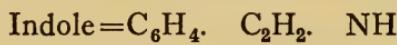
A = acid; AA = acids

	Preparation, etc. page	Effects on plants, etc. page	Solubilities	M.P., °C
Benzoyl-glycine				
R.CO.NH.CH ₂ .COOH (hippuric A)	71, 73	—	A;W	187
— oxide				
(R. CO) ₂ O	—	87	f	—
— peroxide				
R. CO. O. O. CO. R	—	87	f	—
Cinnamic AA				
R. CH ₂ :CH. COOH				
— ordinary (<i>trans</i>)	—	84	W	133
— triglyceride	81	—	?f	111
— <i>allo-</i> (<i>cis</i>)	99	84, 86	—	68
— <i>cis</i> -o-methoxy A	—	84	—	—
— <i>trans</i> -o-methoxy A	—	84	—	—
Hippuric A				
(see benzoyl-glycine)				
Homogentisic A	74, 75	64, 122	b; c; w; a; e	152-154
Hydroxy-phenyl-acetic A	65, 74	65, 74	—	—
Hydroxy-phenyl AA	74		—	—
(general)				
Phenaceturic A				
(see phenyl-glycine)				
Phenol, urinary	72	59	—	—
Phenolglucuronic A	72		—	—
Phenyl-acetic A	73, 74, 98,	13, 28, 9,	w; W	76
R. CH ₂ . COOH	99	32, 33, 37, 39, 56, 86, 99, (112, esters)	—	
— glycerides	81	81	f	oil
— acetyl-glutamine	73	—	—	—
— glycine	73	74	f; a; A	146?
(phenaceturic A)				
— alanine	65, 73, 74	65	—	—
— butyric A	21, 85	33, 85, 86	(various	isomers)
— propionic A	73, 74, 99	33, 34, 39	—	
— triglyceride	81	—	f	oil
— pyruvic A				
R. CHOH. COOH	121	121		
P-phenylenediamine	—	45	—	—
Tyrosine (<i>l</i>)	64, 75, 107	64, 65, 87	a; e; w	270 decomp.
Tyrosol	107	—	—	—

Chemical tests for some phenyl compounds will be found
on pages 121-122.

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Indole, and Compounds



	Preparations, page	Effects on plants, page	Chemical tests, page	Solubili- ties	M.P., °C
Indole	109	86	—	w; a; b; e	52
-bz-substituted cpds.	100, 108, 109	108	—	—	—
-carboxylic cpds.	107-110	—	—	—	—
-esters	100, 108, 109	84, 111, 112, Fig. 3	—	—	—
-2-acetic compound	107	—	—	—	—
-3-acetic A	73, 75, 88, 98-108, 113, 126, 127 (100, isomer)	6-9, 10 seq., 31, 37-43, 46 seq., 51 seq., 60, 77 seq., 86, 93	123 seq.	w; a; c	164-5* 167-8*
-3-acetonitrile	113	113	—	f	B.P. 160 (0.2 mm.)
-3-acrylic A	87 (Bauguess and Berg, 1934)	—	—	w; a; e; W	195-196
-3-alanine (see tryptophan)					
-3-butyric A (normal)	105	12, 28, 29, 32-34, 40, 41, 46, 48, 53, 78, 79, 85, 111	126	a; ?f	123-4*
-3-ethyl alcohol (see tryptophol)					
-3-ethylamine	113	—	—	w; f; a	146 ?
— hydrochloride	—	—	—	—	251-3*
-3-lactic A (dl)	87	87	—	—	144-145
-3-methylacetic A	102, 104	87 (Kögl, 1938)	—	—	—
-3-methyl-indole (see skatole)					
-3-propionic A (normal)	102, 104, 105, 108, 127	26, 31, 32, 33, 40, 78, 79	126, 128	dil. a	133-4*
-3-pyruvic A	104, 105	84	—	—	211 indef. ?
-3-succinic A	107	107	—	W	198 decomp.
-3-valeric A (normal)	108	108	—	B	105*
Indoxyl	75	75	—	—	—
Indylene compounds	101	—	—	—	—

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Indole and compounds—contd.

	Prepara-tions, page	Effects on plants, page	Chemical tests, page	Solubil- ties	M.P., °C
-1:3-diacetic A	105	107	—	a; e; m; W	242*
<i>Iso</i> -indolinone-3-acetic A	—	86	—	—	—
1-methyl-R-3-acetic A	106, 110	84	—	—	—
3-methyl-R-1-acetic A	107	107	—	—	—
5-methyl-R-3-acetic A	—	84	—	—	—
<i>Neo</i> -indole(?) and compounds	109	—	—	—	—
Skatole and cpds.	75, 101, 103, 110	33, 75, 87	—	a; f (skatole)	95 (skatole)
Tryptophan (indole-alanine)	76, 101, 103	—	76, 126 128	—	—
Tryptophol	107, 109	—	—	dil. a ; f	59*

NOTE.—For absorption spectra of some indole compounds, see Hare and Kersten (1937, F); Ward (1923, H; and *ibid.*, p. 907); and Ellinger, in Abderhalden's *Handbuch*, Abt. I, Teil 7, p. 805.

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Naphthyl Compounds

	Preparation, page	Effects on plants, page	Solubilities	M.P., °C.
Naphthyl-acetic AA	111, 113	112	—	—
—esters	30, 54, 112, Fig. 2	—	—	—
— α A	111	12, 28, 29, 30, 34, 37– 39, 41–43, 46–48, 55, 111	sl. W; a ; b ; e	131
— β A	111	Now unim- portant com- pared with — α -A	sl. W; a ; c ; s	142
— α -acetonitrile	102, 111	28, 112	a ; b ; t	79–81
—glycollic AA	111	112	—	—
— α -glyoxylic A (glyoxalic)	111	112	W	98
— ester	111	111	—	—
Naphthoxy-acetic A, β	119	119	—	—

Acetic Acid Derivatives of Other Aryl Compounds

	Preparation, or formula, page	Effects on plants, page
Acenaphthene, $C_{12}H_{10}$ (ethylene-naphthalene, $C_{10}H_8$: C_2H_4)	102, 112	29, 112
Anthracene, $C_{14}H_{10}$	102, 112	29, 112
Benz-furan (see coumarone)	82, 83	82, 83
Coumarone	103, 112	29, 112
Fluorene, $C_{13}H_{10}$	82, 83	82, 83
Indene, C_9H_8	82, 83	82, 83
Thionaphthene (thio-indene?)	82, 83	82, 83

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¹It is not certain whether this entry refers to one or two authors.

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²Correct attribution between these is sometimes difficult to attain.

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